EVIDENCE OF THE MORPHOLOGICAL RANGE, TRANSITION AND EVOLUTION OF STOMATAL PROTECTION MECHANISMS IN SOME SELECTED PROTEACEAE

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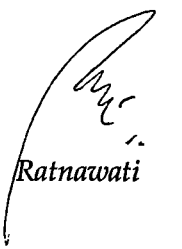

Ratnawati

I dedicate my work to my beloved husband, Agung, and my sons, Odit and Yusta, for their spiritual support during my study.

Abstract

Xero- and scleromorphic adaptations are obviously shown by Australian plants, in response to the Australian climate and edaphic factors. Since these adaptations overlap, there are problems separating the two. Some qualitative hypotheses about the distinction between xero- and scleromorphic characters have been proposed. This research is an effort to quantitatively determine xeromorphic characters in some members of the Proteaceae, in order to elaborate upon some of the existing hypotheses about these characters.

Twenty three species of *Banksia*, 16 species of *Grevillea* and 6 species of *Orites* were sectioned and observed under the light micrscope and measurements were made of the stomatal depressions, margin recurvations, cuticle thickness and hair dimensions. Cuticle peels were made in order to count the number of stomates and hairs on the leaf surface. From the characters measured the following were calculated: the depression index (Id), recurved margin index (Irm), epidermis index (Ie), hair index (Ih) and cuticle components. The sum totals of the calculations were called the stomatal protection component (Spc). These data were then analysed using an analysis of variance with five replicates per species, in order to investigate whether there were any differences in characters determined among species observed. Every index calculated (Id, Irm, Ih, Ie) and the cuticle components were analysed separately, with the aim of determining the intensity of the association between the characters observed and the minimum of the range of habitat annual rainfall, the maximum of the range of habitat annual temperature, the maximum of the range of habitat daily radiation and the maximum of the range of maximum daily wind run. An analysis of correlation was applied to these data. A similar method was applied for observations on juvenile and adult leaves. On the basis of these results the possible evolution of *Banksia* is reconstructed.

Results showed that in *Banksia* species stomatal depression seems to have an important role protecting the stomates from the effect of habitat climatic factors, especially from temperature and radiation effects. The presence of recurved margins and hairs, however, were not as important as stomatal depressions. In *Grevillea* species, cuticle characters had a significant role in stomatal protection, particularly the stomatal and lower epidermis cuticle. A contradictory finding to that of *Banksia* in this genus was that stomatal depression did not contribute to the stomatal depression. In comparison to the two genera already discussed, *Orites* was quite different. However, the hairs had a significant contribution in protecting stomates from temperature and radiation effects.

The phenomenon of leaf transition from juvenile to adult in some species observed showed some performance similarities to plants grown in harsh environments. *Banksia blechnifolia* leaves exhibited more intense stomatal depression and denser leaf hairs in adult leaves. It was assumed that these structures increased stomatal protection from excessive radiation. Similar growing phenomena were displayed by leaves of *Grevillea pyramidalis*. Stomatal depression in this species developed from being not present to being present and then deepened in later leaves. In addition, leaf hairs showed a gradual alteration in density from seedling through juvenile and adult leaves. However, leaves of *B. marginata* did not show the presence of stomatal depressions either in juvenile or adult stage. The leaf hairs, however, exhibited similar phenomena to those found in *B. blechnifolia* and *G. pyramidalis*.

The results suggest that stomatal depression, margin recurvation, leaf hairs and cuticle characters increase stomatal protection either individually or collectively. It thus can be concluded that there is a possibility that these characters are xeromorphic when expressed by *Banksia, Grevillea* and *Orites* species.

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Chapter 1

General Introduction

1. Proteaceae

The Proteaceae is one of the most significant angiosperm families in the Southern Hemisphere (Wrigley and Fagg 1991, Figure 1.1). Its species display a great variety of forms, leaf shapes, colours and flower types. Most are trees or woody shrubs, but some are undershrubs that are rarely woody (*e.g.* some *Conospermum* spp). Alternate and scattered leaf arrangements are commonly found, however, in some genera, opposite (*e.g Xylomelum*) and whorled (*e.g. Banksia* spp.) leaves may be found as well. Common features include simple or compound, entire, toothed or variously lobed leaves, always with no stipule. Flowers are commonly found as racemes or spikes, and sometimes compressed into heads (*e.g. Dryandra*), cones (*e.g. Banksia, etc.*), and corymbs (*e.g.* some *Conospermum* spp.) or they maybe born axially or terminally and solitary.

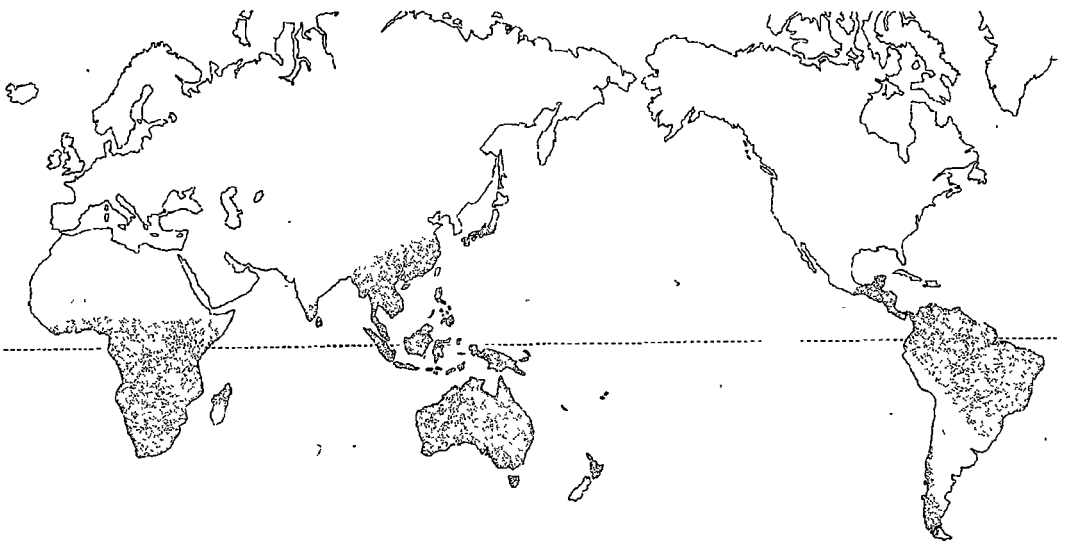


Figure 1.1. The distribution of Proteaceae (After Wrigley and Fagg, 1991)

Apart from the species growing in tropical and sub-tropical areas (*e.g.* Southeast Asia and Central Africa), most Proteaceae inhabit infertile sandy soils and lateritic gravels. The greatest variety of proteaceous species is found in Western Australia where low annual rainfalls (Figures 1.5 and Table 2.2 and 2.3) deep sand, sand over clay or limestone, and laterite dominate soils in these areas. According to Lamont (in Wrigley and Fagg, 1991), up to 14 Proteaceae species per 100 m^2 occur in such areas. Sandstone or sandy soils leached from sandstone are also ideal habitats for Proteaceae growing in eastern Australia. Soil types that do not support many

proteaceous species are limestone and basalt, although there are also a few species in eastern Australia that are reasonably common on basalts.

This study covers three genera of Proteaceae namely, *Banksia, Grevillea* and *Orites. Banksia* are woody evergreen plants. The species habits vary from prostrate to trees up to 25 m in height. Leaves of *Banksia* are hard and tough, prominently toothed and lobed, and often undulating. Adaxial surfaces are usually smooth, but the abaxial surface has a conspicuous midrib. Some species have recurved leaf margins. New leaves are usually covered by dense and brightly coloured leaf hairs. *Banksia* species inhabit areas with a wide range of annual rainfall (Figure 1.5), and from desert to rainforest areas (Figure 1.2) (George, 1994).

Grevillea species are woody plants, with the habits varying from prostate through shrubs, to the tall trees which inhabit tropical rainforests. Leaves are arranged alternately and the shapes are diverse; some are small and entire, others lobed, pinnate or bipinnate. The leaf laminas have the abaxial surface with attractive velvety two-branched hairs. *Grevillea* species occupy habitats with a wide range of annual rainfalls (Figure 1.5), from desert to tropical rainforest areas (Figure 1.3) (Wrigley and Fagg, 1994).

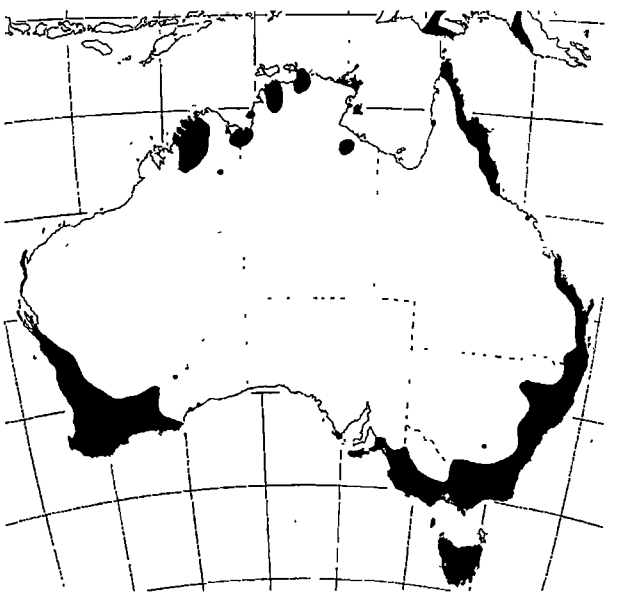


Figure 1.2. Distribution of *Banksia* species (dark areas). Most of the species are concentrated in the south-west and neighbouring areas of Western Australia, in which 58 species can be found. Fourteen species occupy south-eastern and eastern Australia, and two occur in Tasmania (After George, 1994).

Figure 1.3. Distribution of *Grevillea*. About 98% of *Grevillea* species are endemic to Australia. The remaining species are distributed in Papua New Guinea (1 endemic species and 2 Australian species), the Celebes (1 species) and in New Caledonia (3 species) (After Olde and Marriot, 1994).

Figure 1.4. Distribution of *Orites* species. Eight species are distributed in Australia; four are endemic to Tasmania and the remaining species occupy alpine areas of New South Wales and Victoria (After Wrigley and Fagg, 1994).

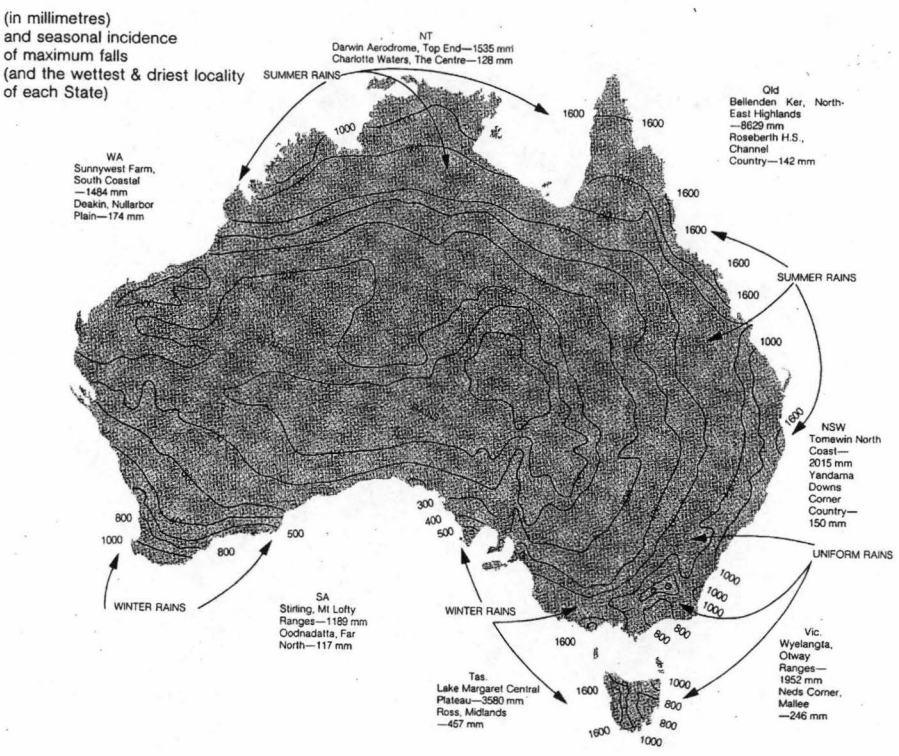


Figure 1.5. Average annual rainfalls in Australia (After Read, 1994).

Orites is a small genus with eight species and possibly another undescribed species in north Queensland. All species are woody plants, with habits varying from low shrubs to forest trees. Leaves are arranged alternately, the shapes are very diverse from needle shaped (*O. acicularis*) through revolute margined (*O. revoluta*), to broad lamina (*O. diversifolia*). Leaves with a broad lamina may have entire, toothed or lobed margins. This genus inhabits two different ecological niches. The southern species occupy alpine areas and usually have a shrubby habit, while the northern species are trees found in rainforests. *Orites diversifolia* is an exception because it occupies both areas (Figure 1.4) (Wrigley and Fagg, 1991).

2. Xeromorphy

Hill (1998) stated that typical xeromorphic characters are those that are capable of increasing the boundary layer, while scleromorphy is defined as a morphological response to low nutrient level, especially phosphorus (Beadle, 1968) and manifests itself in many different ways. These morphological responses are common in Australian native plants, because they were adapted to Australian physical factors a long time ago. Consequently, it is confusing when no obvious line separates the characteristics of xeromorphy and scleromorphy.

The boundary layer involves structures that can keep stomata in positions to avoid excessive transpiration, *e.g.* in a deeper position than the epidermis, or in a hidden position among hairs. Structures that can increase the boundary layer include abundant trichomes, leaf depressions, revolute leaf margins, stomatal plugs (Hill, 1994) and thick cuticles (Duddington, 1969; Cowan, 1977; Johnson, 1980; Rudall, 1980). Among these characters, trichomes (hairs) are interesting because they have several roles and sometimes the roles are contradictory. An interesting example is the finding of Brewer and Smith (1997) that leaf trichomes of montane and alpine plants have a role as a water repellent. On the other hand Duddington (1969) noted that there is a positive correlation between cuticle thickness and rainfall.

The boundary layers could be qualitatively and quantitatively different among species in the same genus. The diversity is interesting because it is assumed to reflect habitat conditions. Sometimes the difference is subtle and may be quantitatively different, *e.g.* hair size, depression dimension, *etc.* In some species the differences occur between leaf life history, for example, between juvenile and adult leaves. The level of differences can be low or high depending on, as Allsopp (1965) suggested, genetic make-up and the magnitude of the environmental pressure the leaves experience during their life history (Allsopp, 1965). Structural differences can also be observed after millions of years through a long period of evolution. This evolution took place very slowly and is assumed, by some investigators, as a response to the climatic changes (*e.g.* Beard, 1977; Hill and Christophel, 1988; Jordan, 1995)

This research concentrates on some Proteaceae taxa, namely species of *Banksia*, *Grevillea* and *Orites*. From these taxa, attention is focused on the leaf structures, and particularly on the stomatal protection mechanisms. This research examines the dichotomy between xeromorphy and scleromorphy. It is expected that the results of this study will shed light on this problem. This study was carried out to ascertain whether there is a transition in the stomatal protection mechanism between juvenile and adult leaves and to provide data to assist in explaining the morphology observed in fossil Proteaceae leaves.

Chapter 2

The Morphological Range of Stomatal Protection Mechanisms in Some Selected Proteaceae

2.1. Introduction

The nature of plants, as living things on the Earth, is continually modified by reproduction and adaptation to their environment. The adaptation of plants can be structural or functional, and these often take place in parallel. That is, one causes the other, or vice versa.

Structural modification, as noted by Esau (1977), is reflected in different forms. Esau (1977) showed that certain plants growing in a dry place produce particular accessories to protect the aerial parts from evaporation, while others produce underground water storage organs or deep roots. Structural modification is also reflected by plants growing in different environments, generally stated as "evolutionary adaptations to the conditions of the specific habitat" (Esau, 1977).

Plants have particular morphological characters considered as structural modifications to the amount of water in the environment. In dry conditions, lack of water is the limiting factor for plants to survive. In such dry conditions plants have mechanisms to obtain water, maintain their water content, and stay alive (Treshow, 1970).

Plants require certain structures to support to the mechanisms utilised for coping with dry conditions. In other words, plants modify their structure into a xeromorphic one, and thus are called xerophytes. However, according to Treshow (1970) xeromorphism can be exhibited by a non-xerophyte when exposed to water stress conditions. On the contrary, a xerophyte might not have xeromorphic characters as they are normally defined, because it is unnecessary to have such structures (for example ephemerals that survive dry conditions in a resting phase) (Duddington, 1969).

There are many xeromorphic characters. These include the formation of thick wax, functioning as a protector of excessive transpiration (Treshow, 1970), a thick and compact mesophyll, hairy leaf surfaces, abundant stomates, *etc.*

Structural Modifications in the Leaf System

Leaves, as one of the aerial parts of plants, receive direct environmental effects from their habitat, especially the atmosphere. This is exacerbated by the fact that leaves have the largest surface area:volume ratio of all above ground organs. The existence of stomates as the gas exchange point of the leaves increases the impact of atmospheric effects on leaves. Also, leaves, as the final destination of raw materials from roots, are affected by soil environments. This is especially true in water stress conditions, when leaves suffer effects from the atmospheric and soil habitats. These conditions are in accordance with Fahn's (1982) assumption that the adaptation of plants in certain environments may mostly be shown in leaf structure (Romberger *et al.*, 1992).

Plant adaptation is a variable phenomenon, since it relates to many conditions, such as sensitivity of plants, the quality and quantity of the stress factors and the condition of other habitat factors. Thus, these plastic responses of plants (Rapson and Wilson, 1992) to a certain stress factor are also variable. Moreover plastic responses are very significant responses to environment, even though they utilise more energy than the genetic responses (Rapson and Wilson, 1992).

Although there are differences in response to environmental stress, there are common responses reflected in leaves. The common characteristics of leaves living in water deficit conditions were proposed in 1904 by Zalenski, as the so-called Zalenski Law (Maximov, 1931 in Stocker (1960)). These characteristics include a reduction in the size of the epidermal cells, hairs, stomates and mesophyll cells, and the extent of the spongy parenchyma and the intercellular spaces. On the other hand, there are increases in the length of the veins, the number of stomates and hairs per unit area of leaves, of the thickness of the outer cell walls of the epidermis, and the development of a multi-layered palisade parenchyma. Such characteristics are expressed by plants grown in dry air, sunny places and by the upper, compared with the lower, leaves of the exposed plants (Stocker, 1960).

Fahn (1982) demonstrated that xeromorphic leaves are usually rather thick and leathery, with well developed cuticle and abundant hairs. Epidermal and sub epidermal cell walls are usually lignified, mesophylls are differentiated into spongy and palisade tissues, with the latter tissue more abundant than the former. The abundance of palisade tissue is more obvious in leaves exposed to high light intensity. The hypodermis, a layer below the epidermis, might be present. The

vascular system and sclerenchyma are well developed, however, according to Hill (1995) these characters are also well developed in scleromorphic plants. In addition, xeromorphic leaves also have narrow surface areas, sometimes with cylindrical or rolled shapes. These shapes protect stomates from environmental factors.

Oppenheimer (1960) gave a clear example of the effectiveness of rolled leaves in reducing transpiration during dry conditions. He found that transpiration could be reduced as much as 46 to 63% by the rolling of leaves of grasses in the Mediterranean region and in Auvergne. Similar effects were also found in certain grasses in the Sahara. However, in many desiccation resistant species, leaves do not roll even though the water content achieves lethal levels (Kozlowski, 1968).

Other leaves show different responses to a dry environment. Rudall (1980), in her observations of the leaves of the subtribe *Hyptidinae* of the Labiatae, found that the plants showed many of the common characteristics of xeromorphism, and also reflected other characteristics, namely abscission of leaves in the dry season and reduction of number and size of hydathodes.

1. Stomates

A stomate is a pore or mouth (Greek) in the epidermal tissues. The structure consists of a pore directing to a substomatal chamber, as a continuation of intercellular spaces, and two guard cells having a role in opening and closing mechanisms of the pore or aperture. In many plants these mechanisms are supported by subsidiary or accessory cells directly attached to the guard cells (Esau, 1977).

Stomates are commonly found in green aerial parts of plants, especially in leaves (Esau, 1977). Other parts of plants usually having stomates are green stems, petals, sepals, *etc.* Leaves can be classified into three categories according to the presence of stomates on leaf surfaces, these are amphistomatic (stomates on both surfaces); hypostomatic (stomates on the lower (abaxial) surface); and epistomatic (stomates on the upper (adaxial) surface) (Esau, 1977).

The number of stomates is variable depending on habitat conditions (Duddington, 1969; Meyer *et al.*, 1973; Esau, 1977; Reihman and Schabilion, 1976; Montenegro and De La Puente, 1977; Christodoulakis and Bazos, 1990; Christodoulakis and Fasseas, 1991; Abrams *et al.*, 1994). However, the number of stomates is not by itself an

reliable indicator of a plant's adaptation to a dry or wet environment (Sunberg, 1986). Volkens' comment is supported by a number of opinions and results published in recent literature and research reports. Some researchers have assumed that in dry conditions there is a decrease in stomatal density (Duddington, 1969; Montenegro and De La Puente, 1977; Christodoulakis and Bazos, 1990). On the other hand, others have observed the opposite effect, that there was an increase (Esau, 1977; Michener, 1981; Sundberg, 1986; Christodoulakis and Fasseas, 1991; Abrams *et al.*, 1994). The recent literature attempts to explain this conflicting view about stomatal density in xeromorphic leaves, and Noggle and Fritz (1976) give one such explanation. According to them, the greater the stomatal frequency, the smaller the internal resistance of the leaf. It would appear that the more stomates per area of leaf surface there are, the higher the rate of water vapour loss.

A different finding on *Sinapis alba* grown in moist (55%) and dry soils (25%) was recorded by Ripple (1919, in Stocker (1960)). In dry soils the number of stomates per square mm (143.1) was greater than that in moist soils (84.3). Furthermore, the stomatal number on the lower surface was also much greater (357.8 in dry soils and 276.7 in moist soils) than that on the upper one (143.1 in dry soils and 84.3 in moist soils). In that case, there was an increase in the number of stomates in dry soils; it thus agreed with the Zalenski Law (Stocker, 1960). Yet, the fact is that in the lower surface, which was considered to be moister than the upper one, a greater number of stomates were present than that of the upper surface.

Since the function of stomates is to exchange carbon dioxide, oxygen and water vapour between plant parts and their habitats (Levitt, 1974), their structure and position have roles in determining the amount of gas exchanged (Devlin, 1966). Furthermore, Brown and Escombe (1900, in Devlin (1966)) concluded that "diffusion through a small circular pore is more nearly proportional to the perimeter or diameter of the pore than to its area".

Several researchers (summarised by Devlin (1966)) also investigated Brown and Escombe's conclusion. Sayre (1926, in Devlin (1966)), in his experiment on leaf transpiration, also found that the wider the stomate pores, the higher the water vapour loss (Table 2.1).

Table 2.1. Diffusion of water vapour through small openings under uniform conditions.*

Diameter of pores (μm)	Loss of water Vapour (μg)	Relative amount of water loss	Relative areas of pores	Relative perimeters of pores
2.64	2.655	1.00	1.00	1.00
1.60	1.583	0.59	0.37	0.61
0.95	0.928	0.35	0.13	0.36
0.81	0.762	0.29	0.09	0.31
0.48	0.455	0.17	0.03	0.18
0.35	0.364	0.44	0.01	0.13

* After Sayre (1926, in Devlin (1966)).

According to Noggle and Fritz (1976), there are two limiting factors associated with transpiration. These are "the internal resistance (stomatal resistance)" and "the resistance external to the leaf". Both of these determine the "transpirational flux" defined as the amount of water vapour transpired (μg) per square centimeter of leaf surface per second. The stomatal resistance itself is determined by many factors, namely stomatal frequency, the shape and size of the substomatal cavity and the stomatal pore, and the size of the stomatal opening. Among those factors, however, the size of the stomatal opening associated with the turgidity of guard cells, basically determines the internal resistance.

Bange (1953, in Noggle and Fritz (1976)) gave a clear graphic illustration of this (Figure 2.1). The graph shows that whether in moving or still air, the transpirational flux tends to increase as the stomatal aperture (opening) increases. In moving air, the transpirational flux gradually increases. However, in still air it increases more slowly. These phenomena closely relate to the density of water vapour above stomates, where in moving air the water vapour is blown away relatively quickly. On the other hand, in still air the water vapour collects above stomates, in a boundary layer.

A similar opinion to Esau's was proposed by Meyer *et al.* (1973), that the rate of transpiration is not determined by the size and distribution of stomates, but by other factors. However, they did not specify which other such factors were more important. It can thus be assumed that in dry habitats, plants tend to have smaller, hidden and fewer stomates in order to reduce transpiration. Many observations

support this tendency, but others show different results. The following discussion provides details of these observations.

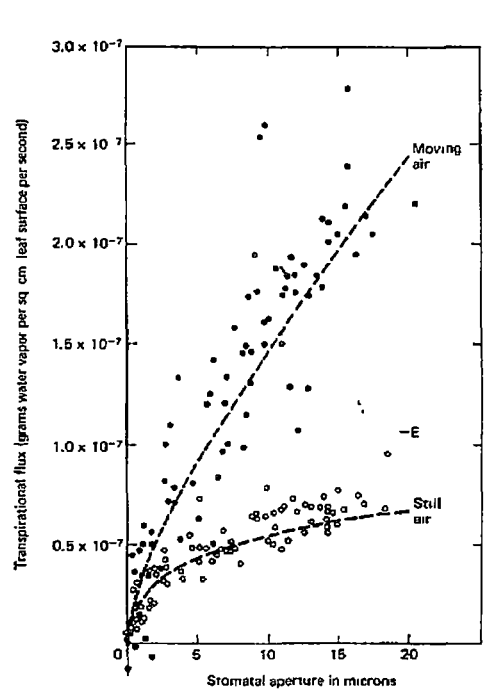


Figure 2.1. The relation between transpiration flux and stomatal aperture in *Zebrina pendula* in still air (open circles) and moving air (black circles). Theoretical values are indicated by dashed lines. After Bange (1953, in Noggle and Fritz (1976)).

Duddington (1969) explained that positioning of the stomate below the normal epidermis level provides a space above it, protecting water vapour from wind. As a consequence, the space is saturated and functions as a barrier to further transpiration. This accords with Esau's view. Esau also gives some examples: in *Nerium oleander* stomates are in cavities (stomatal crypts), and in *Ericales* they are found in grooves similar to those in *Alethopteris lesquereuxi* (Reihman and Schabilion, 1976). Rudall (1980) observed xeromorphism in the leaves of Hyptidinae (Labiatae). The results showed that in this taxon stomates were sunken in the epidermis or in the abaxial interveinal depressions. The sunken (depressed) stomates were also observed in some Middle Eocene fossil and recent *Lauraceae* members. In this taxon the depressed stomates were protected by scales (Bandulska, 1924). Other observations conducted on the fossil leaves of *Frenelopsis varians* from the Lower Cretaceous of Central Texas (Daghlian and Person, 1977), indicated that there were sunken stomates.

Besides being hidden, xeromorphic stomates also tend to locate themselves in special shaded places, such as on the abaxial surface. This condition can be observed in *Proteaceae,* which has members with hypostomatic leaves (Carpenter, 1994). The same kind of stomates were also found in the xeromorphic summer leaves of *Thymus capitatus* (Christodoulakis and Bazos, 1990) and in some members of *Banksia* (Figure 2.2) (Hill, 1994). In several *Banksia* species; e.g. *B. grandis, B. marginata,* and *B. chamaephyton,* stomates are not only located on the abaxial leaf surface, but are also laid within a range of leaf depressions including "large balloon-like pits" (Hill, 1994) and thus they are very protected. Other *Banksia* species with narrow leaves, such as *B. spinulosa, B. ericifolia* and *B. grossa,* protect stomates by abaxial-inrolled leaf margins and trichomes. The very inrolled leaf margins form tapering cavities with dense hairs along their edges. Other hidden stomate locations were observed in *Allocasuarina littoralis, Allocasuarina torulosa,* and *Casuarina equisetifolia* (Figure 2.3) (Hill, 1994). In these species stomates are laid along cladodia pits and also protected by trichomes.

A different strategy was reported for *Keckiella* (Scophulariaceae) which has dense stomates on the upper surface (Michener, 1981) and also in *Phlomis fruticosa,* a seasonally dimorphic species which, when growing under a controlled environment, has amphistomatic leaves in winter and summer (Christodoulakis and Fasseas, 1991). However, the wild type has hypostomatic leaves (Christodoulakis, 1989 in Christodoulakis and Fasseas, 1991). This demonstrates that a wide variety of morphological responses are found in response to similar xeric stresses.

Guard cell characteristics indicating xeromorphism have been observed. Johnson (1980) counted the ratio of the length to the width of the guard cell pairs in *Leptospermum* (Myrtaceae). The results showed that in this genus, especially in the Australian material of *Leptospermum flavescens,* the ratio was slightly lower than average in more xeric conditions. This result is in agreement with the findings of Maximov (1931, in Bongers (1973)) and other workers, who showed that xeric plants tend to have more circular stomates (Johnson, 1980).

Despite the lack of changes in stomatal index, Niemann (1932, in Stocker (1960)) concluded that the number of stomates increased in dry conditions and it varied among individual species. Even in the same plant, the stomatal density varies from the upper to the lower surface of the leaf of the plant (Farkas and Rajháthy, 1955 in Stocker, 1960). Farkas and Rajháthy conducted research on flax, oat and barley plants grown under dry, normal (control) and irrigated conditions in Hungary. Their findings (Fig. 4) were in accordance with Niemann's finding that even though the

plants were given the same treatment the stomatal density varied among species. Moreover, the Zalenski Law was valid in respect to the greater number of stomates on the upper leaf surfaces compared with the lower ones. The greater number of stomates was in parallel with the reduction of the length of the stomates (Stocker, 1960). Similar experiments were carried out by Kokina (1926, in Stocker (1960)), Lundkvist (1955, in Stocker (1960)) and Abrams *et al.*, (1994).

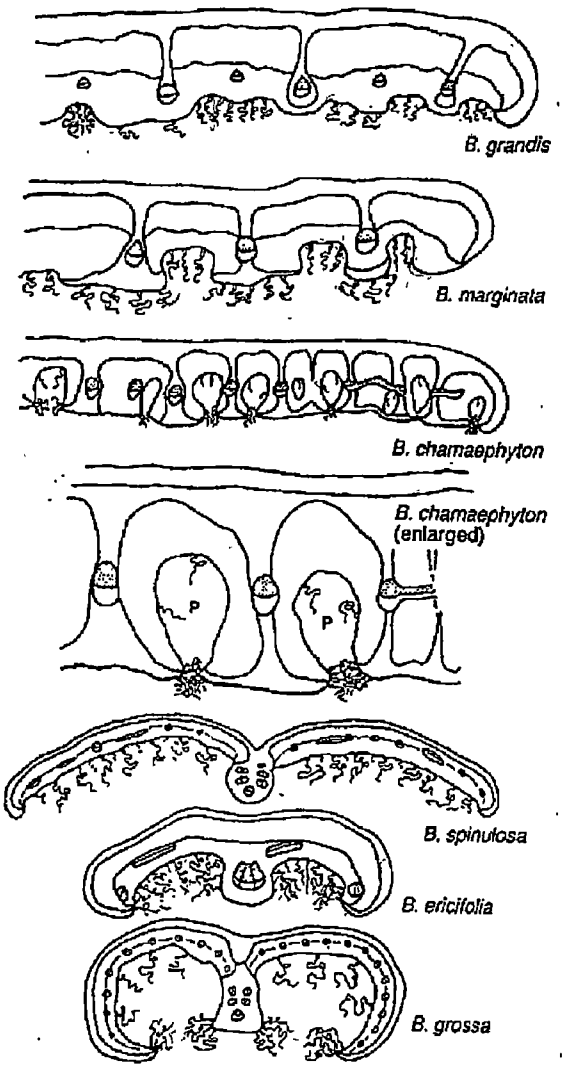
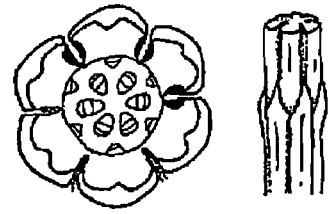
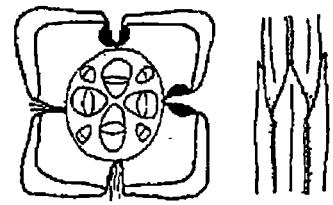


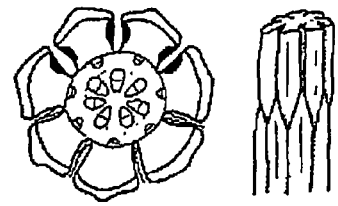
Figure 2.2. Cross section of some *Banksia* leaves. In the top three species, stomates are laid in a range of leaf depressions and also protected by hairs. In the bottom three species, stomates are protected by a range of abaxial-inrolled leaf margins and hairs. After Hill (1994). p= stomatal pit.



Allocasuarina littoralis



Allocasuarina torulosa



Casuarina equisetifolia

Figure2.3. The cross sections of cladodia of some Casuarinaceae species. Stomates are located along cladodia pits (black spots, only shown for some of the furrows) and also protected by trichomes. After Hill (1994).

Qualitative figures on the influence of soil moisture on the structure of bean *(Phaseolus)* leaves were presented by Kokina (1926, in Stocker (1960)). The bean leaves grown in dry soils showed denser and smaller stomates than those in the moist soils. These characteristics were not always expressed by plants grown in a water deficient habitat. Plants grown in soils with excessive water show the same changes in the stomatal dimension ratios as in water deficient soils (Lundkvist, 1955 in Stocker, 1960). This finding suggested that xeromorphism was not always due to the lack of water. Another factor which can change mesomorphic leaves into xeromorphic ones is lack of nitrogen (*e.g.* Kraus and Kraybill, 1918; Mothes, 1932; Müller-Stoll, 1947, all in Stocker, 1960). On the contrary, excess nitrogen also produces xeromorphic effects (Stocker, 1960). In addition, Wöstmann (1942, in Stocker (1960)) reported that Ca produced xeromorphic effects in respect to the

thickening of the cuticle and lowering the stomatal position below the epidermis surface. The lack of phosphorus also caused the expression of xeromorphic characters as proposed by Beadle (1968). Finally, Biebl (1956, in Stocker (1960)) reported that irradiation in the early development of plants produced xeromorphic and succulent characters.

Abrams *et al.* (1994) compared the guard cell length of plants living in 3 sites; xeric, mesic and wet-mesic. The average guard cell length of the plants was 25.9 ± 0.68μm for those living in the xeric site, 22.34 ± 0.34μm for those in the mesic site and 18.78 ± 0.57μm for those in the wet-mesic site. Furthermore, they found that in the xeric site the stomatal density was 279.9 ± 9.4, in the mesic site it was 237.8 ± 13.3, and in the wet-mesic site it was 271.8 ± 9.4. Thus, the stomatal density in the xeric site was the highest, but there was no tendency for a decrease in stomatal density from the mesic to the wet-mesic site. This result is in accordance with Volkens' (1887, in Sunberg (1986)) suggestion that there is no correlation between stomatal density and environment. This is a different tendency, however, from that found in succulent and non-succulent desert plants by Sunberg (1986). His findings indicated that there was a tendency for stomatal density to reduce with increasing guard cell length.

Fahn (1982) observed wall thickenings in guard cells. His study indicated that at the end of the summer, in some desert plants, there will be some wall thickenings in the guard cells. This condition appears to be associated with excessive loss of water avoidance, that is by closing stomates. This is in accordance with results from several researchers suggestion that during critical drought periods the thick-walled guard cells keep closing (Fahn, 1982).

Finally, even though there are many observations of the morphological characteristics of stomates of xeromorphic leaves, some results contradict others, and therefore it is important to consider the presence of another structure, that is the cuticle. This structure is important since it is also the alternative water passage to the atmosphere. Therefore, in some species when stomates close, transpiration still occurs.

2. Cuticle

Zeiger *et al.* (1987) noted that cuticle is a structure that has a primary function as a transpiration protector, therefore, it has to have a special character *i.e.* low permeability to water vapour (Zeiger *et al.*, 1987). In order to support that function, structurally and chemically cuticle consists of many layers and contains many kinds of chemical substances. It lays directly on the surface of epidermal tissues, exactly on

the outer part of a "pectin-rich layer", as the transition area. From the innermost to the outermost part cuticle consist of a cuticular layer, containing polysaccharides, pectin, a small amount of cellulose and cutin, cuticle proper contains wax crystals and it is covered with an epicuticular layer containing crystalline or shapeless wax.

Since the cutin and wax layers are continuous and very hydrophobic, these support the epidermal layer in protecting its water content, water absorption, and water containing material (Romberger *et al.*, 1992). The ways that the epicuticular layer were accumulated and deposited affect its effectiveness in limiting transcuticular water and solute flows (Martin and Juniper, 1970; Romberger *et al.*, 1992). According to Martin and Juniper (1970) generally, 5-10% of the whole transpiration is cuticular transpiration.

It is generally accepted that there is a correlation between cuticle thickness and dry habitats (Duddington, 1969), so the drier the habitat the thicker the cuticle. The results of many experiments conducted by many researchers show that they are in accordance with this opinion (*e.g.* Johnson, 1980; Rudall, 1980).

However, Esau (1977) mentioned that in dry habitats the thickness of cuticle is variable. This is supported by Martin and Juniper (1970) who explained that many experiments have been done to try to correlate between habitats and the thickness of cuticle and wax. However, some showed contradictory results. Romberger *et al.* (1992) also mentioned that a few xeromorphic plants did not show well-developed cuticle and wax layers. Seemingly, these plants had other mechanisms to cope with excessive water evaporation.

In spite of these controversial opinions on the correlation between cuticle thickness and dry habitat, many researchers continued working in this topic, and most of the results showed that there was a positive correlation between the cuticle thickness and a dry environment (Dickison, 1975; Daghlian and Person, 1977; Montenegro and De La Puente, 1977; Rudall, 1979; Johnson, 1980; Carpenter, 1994). In accordance with the topic, Carter and Brandham (1979, in Rudall (1980)) also observed that on many *Alöe* leaves "the waxy surface bloom" was thicker in the dry season.

Not only do living plants demonstrate this, but extinct ones do as well. Daghlian and Person (1977) worked with stem parts of *Frenelopsis varians* and the results indicated that the cuticle was very thick (15.0 - 26.5μm). The habitat where *Frenelopsis varians* was extant was dolomite-marsh deposits predicted to have an excessive

evaporation. By having a very thick cuticle this species protected itself from excessive loss of water.

Similar experiments on living plants were conducted by other researchers. In several members of the Cunoniaceae considered as xerophytes, Dickison (1975) observed that in combination with leathery texture and abaxial-inrolled margin, leaves also had well-developed cuticle. Rudall (1980) observed the subtribe Hyptidinae (Labiatae), consisting of four small genera and one big genus, *Hyptis*. The structures indicated that most of Hyptidinae members tend to be xeromorphic. However, a few of them had cuticular striations on the leaves of all species members; e.g. *Hyptis* section *Rhytidea, Hyptis* section *Pusillae,* but others that did not or had variable striations, include *Hyptis* section *Hypenia* subsection *Densiflorae, Hyptis* section *Trichosphaeria* subsection *Crinitae,* and *Hyptis* section *Eriosphaeria* subsection *Passerinae.*

Other observations were made by Rudall (1979) on 25 species of *Eriope* (Labiatae). The results indicated that most of the *Eriope* species had xeromorphic characters, especially the presence of trichomes. Certain species, however, which did not have dense trichomes, protected their leaves with well-developed cuticles, "sometimes with cuticular striations and sometimes a waxy surface" (Rudall, 1979) (e.g. *Eriope crassipes* subsp. *cristalinae*). By having these structures, the species with few trichomes could protect their leaves from excessive transpiration. The presence of thicker cuticles was also observed in *Grevillea* and *Banksieae,* both of which were considered as xerophytes living in North Queensland rainforest (Carpenter, 1994). All of the observations were qualitative, but others were quantitative, in order to give clearer data about the correlation between xeromorphic characters and dry habitats.

Montenegro and De La Puente (1977) tried to compare morphological responses of *Colliguaja odorifera* growing in the south and the drier north-facing slopes in Chile. These plants showed obvious differences in leaf structures, especially in leaf thickness. Detailed observations indicated that there were differences in the internal structures, such as cuticular thickness, stomatal numbers, mesophyll thickness, *etc.* The cuticular thickness was remarkably variable, seemingly correlated to a big difference in the humidity between the two slopes. Quantitatively, it could be seen that on the south-facing slope the cuticular thickness was 5.1μm and on the north-facing one it was 8.0μm. Moreover, on the north-facing slope, the cuticular thickness on the upper surface was double that on the lower one. It seems that this phenomenon was mostly correlated with the excessive light intensity in that location. In addition, the function of the cuticle is not only to protect the plant from excessive

transpiration, but also to reflect strong radiation on the leaf surface (Martin and Juniper, 1970).

Although cuticle and wax are prominent structures in xeromorphic leaves, their presence does not ensure the cessation of transpiration (Parker, in Kozlowski (1968)). This conclusion is based on Oppenheimer's (1960) finding that when the cuticle dries out, there is a constriction in the submicroscopic channels that pass through the cuticle but water can still pass through.

3. Trichomes

Another characteristic of xeromorphic leaves is the presence of hairs (trichomes), which protect water vapour from wind (Duddington, 1969), reflect high intensity light (Treshow, 1970; Lyshede, 1976; Ehleringer, 1983), insulate the mesophyll from overheating (Esau, 1977), and absorb water from rain, dew, fog or clouds in a xeric climate (Lyshede, 1976).

Structurally, trichomes can be distinguished by the presence of glands in cells, the number of cells, and the complexity of the structures. Therefore, many trichome classifications have been proposed by many workers, such as Dickison (1975). However, no satisfactory trichome classification based on those characters was produced until 1983 (Behnke in Rodriguez *et al.*, (1983)).

The functions of trichomes in xeromorphic leaves are in accordance with the proper function of the epidermis, that is, as a protective tissue, especially for diminishing the risk of desiccation. However, it does not accord with Johnson's (1975) opinion that only dead trichomes protect leaves from desiccation; living trichomes themselves can lose water. Some researchers report that trichome removal decreases transpiration (Kozlowski, 1968) supports the above idea. Many workers have conducted research in this interesting area (Rodriguez *et al.*, 1983), some of it qualitative and some quantitative. These reports will now be discussed in detail.

A study on the physiological anatomy of some plants (*Casuarina lepidophloia, Geijera parviflora, Pholidia scoparia, Atriplex vesicarium, Ragodia gaudichaudiana,* and *Kochia sedifolia*) from the arid zone of South Australia was carried out by Wood (1923). It is reasonable to study the relationship between the two factors, physiology and anatomy, since they are strongly related. The findings showed that there was a

relationship between the transpiration rates and structural modifications which developed in the transpiring organs. The six species could be divided into three patterns of transpiration curves, each of which was a function of structural characteristics in the transpiring organs.

In *Casuarina lepidophloia,* which fits the first pattern, the transpiration curve closely approximated that of evaporating power during the day. Actually, the stomates were in furrows and protected by few hairs that did not give enough protection from wind and during the night since there was stomatal control, the transpiration curve lacks agreement with the evaporation curve. The second pattern, in *Geijera parviflora* and *Pholidia scoparia* showed an agreement between the maximum transpiration and the maximum evaporation rates, but the transpiration curve was much flatter. It appears from these findings that neither species gave a direct response to fluctuations in environmental conditions. This was supported by the presence of an oily secretion whose vapours inhibit transpiration, of the ridge in *Geijera parviflora,* and also of the scales in *Pholidia scoparia.* The third pattern, in *Atriplex vesicarium, Rhagodia gaudichaudiana,* and *Kochia sedifolia* showed a straight line for transpiration rates. This indicated that these three species had constant conditions that were not affected by external factors. This condition was supported by an air jacket, a thick cover of vesicles or air cells, surrounding the leaf surface. In this condition a regular and equal amount of water was transpired at equal intervals; and then the water vapour was swept away. The greater length of curve parallel to the time axis in *Kochia sedifolia* was caused by the presence of hairs, which as an extension of terminal air cells, had a role in insulation. During the night, since there was stomatal control, the transpiration rate decreased. The increase of transpiration rate from 8 to 9 pm, was probably caused by turgor changes in guard cells or by the change of water vapour pressure in intercellular spaces (Wood, 1923).

Studies on trichomes have been carried out by many researchers, who generally reported that plants growing in dry habitats tend to have hairy or pubescent leaves, while mesic plants had glabrous or glabrate leaves. Moreover, they also explained that in some species leaves were pubescent during wet periods and glabrous during dry periods (Rodriguez *et al.,* 1983). Johnson (1968, in Rodriguez *et al.,* (1983)) found that, even though the incidence of pubescence in four communities (sandy beach, old field, oak forest and red maple swamp) growing in a range of humidity areas was similar (70 - 80% of the species), the denser pubescence was observed in the drier areas.

These findings are in agreement with Duddington's (1969) study that found that in

xeromorphic leaves trichomes are frequent. Trichomes have a significant role in reducing transpiration by protecting the stomatal openings in windy weather. Rudall (1980) reported that branched-hairs were found in many members of *Hyptis, e.g. Hyptis* section *Subumbellaria, Hyptis* section *Rhytidea, Hyptis* section *Buddleoides* and *Hyptis* section *Cyrta* subsection *Lavandulaceae* (Rudall, 1980). Moreover, she also found that xeromorphism in this taxon was reflected by an increasing number of glandular hairs. In Proteaceae, uniseriate 3-celled trichomes were observed and mostly found on the stomate-abundant surface (Carpenter, 1994). Christodoulakis and Fasseas (1991) found denser hairs on both surfaces of summer leaves of *Phloemis fruticosa,* these being more xeromorphic than those of the winter ones.

In *Banksia, e.g. B. grandis, B. chamaephyton,* and *B. grossa,* trichomes are found on the abaxial leaf surfaces. They are concentrated around stomates, in the pit openings or along the mid vein and leaf margins (Hill, 1994). However, in Casuarinaceae, *e.g.* in *Allocasuarina littoralis, A. torulosa* and *Casuarina equisetifolia,* trichomes are in deep grooves along the stem (Hill, 1994) that protect stomates from environmental factors. These findings give a more clear indication that trichomes tend to enclose stomates as closely as possible. Lyshede (1976) observed the structure and function of trichomes in *Spartocytisus filipes.* The trichomes had shiny surfaces which reflected sunrays, and thus protected the chloroplasts from overheating. The cell walls of the trichomes were rich in pectin and also the cellulose microfibrils in the secondary cell wall were parallel to the cell axis. Both conditions allowed water to be absorbed.

To explain more about the role of trichomes in xeromorphic leaves, quantitative experiments have been carried out. Haberlandt (1928, in Devlin (1966)) conducted an experiment to measure the effectiveness of a glabrous surface in reducing transpiration. He used two leaves of *Stachys lanata,* which has very dense hairs on both surfaces. Both abaxial surfaces were painted with cacao-wax, one adaxial surface was shaved and the hairs on the other were untouched. Then the leaves were allowed to transpire for twenty-four hours in a shaded place at a temperature of 20 - 25°C, and exposed to sunlight for one hour (with 20 minutes disturbed by clouds). The results showed that in the shade the ratio of the lost weight of the intact to the shaved leaves was 1 : 1.42. However, in the location exposed to sunlight the ratio of those values was 1 : 2.09. In the second case the hairy surface was more efficient in reducing transpiration, since most transpiration was via the stomates.

A similar experiment was carried out by Ehleringer and Björkman (1978). The subjects were *Encelia farinosa* which has pubescent leaves, and *E. californica*

which has glabrous leaves. Both grew along the aridity gradient in southern California. They compared the leaf absorptance of *E. farinosa* with hairs intact and with the hairs removed. A measurement on the leaf absorptance of *E. californica* was also conducted, and this was compared to *E. farinosa*. The results showed that the leaf absorptance of hair-removed leaves of *E. farinosa* was nearly identical to that of *E. californica* leaves (Figure 2.4). It can be assumed, from the graph, that all species along the aridity gradients have leaves with the same basic absorptance pattern. The differences depend on the various amounts of a blanket reflector (Ehleringer and Björkman, 1978).

A different view about the effect of hairiness in light reflectance in *Gynura aurantiaca* was proposed by Gausman and Cardenas (1969). According to their findings, hairiness increased light reflectance only in the 750 - 1000 mμm region. Based on the results, they assumed that: 1. Discoloration of the stump exudate after shaving increased the leaf absorptance in the 750 - 1000 mμm region; 2. Undoubtedly, hair removal increases water loss in leaves. According to Knippling (1967, in Gausman and Cardenas (1969)) it increased light reflectance, especially in the 1000 - 2500 mμm region; 3. The reflection of light was a case that highly depends on the hair length and the hair arrangement on leaves.

Munz (1959, in Rodriguez *et al.*, (1983)) conducted research on several species of *Salvia*. The research was carried out on *S. mellifera, S. leucophylla* and *S. apiana,* which grew along an increasing aridity gradient in southern California. The leaf absorptance spectrum for these three species was between 400 - 800 nm. The density of trichomes on these species increased as the aridity of the areas increased. The leaves of *S. mellifera* were glabrous, of *S. leucophylla* moderately pubescent and of *S. apiana* pubescent. Figure 2.5 shows that the glabrous leaves of *S. mellifera* absorbed the highest percentage of 400 - 700 nm monochromatic light among the leaves of these three species. The leaf absorptance of *S. apiana* was the lowest and that of *S. leucophylla* was moderate.

Similar data to those of *Salvia* were recorded in *Encelia,* which grew along a similar but more extensive aridity gradient in southern California (Ehleringer, 1980). Billings and Morris (1951, in Ehleringer (1980)) compared the spectral characteristics of species from several communities, growing in different degrees of aridity in the Great Basin Desert. They reported that the average reflectance of leaves increased (absorptance decrease) as the aridity of the habitat increased.

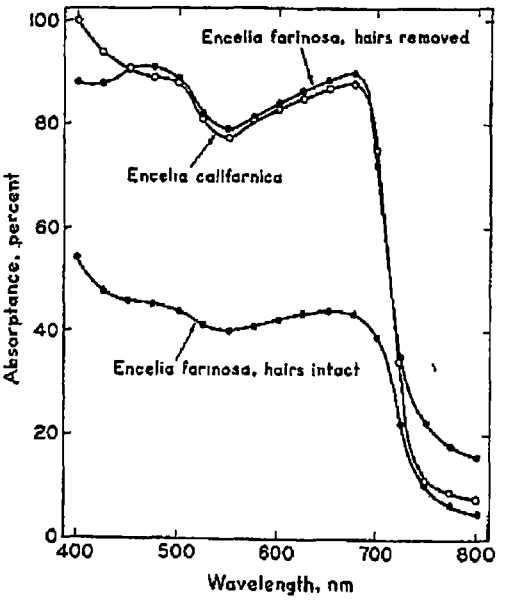


Figure2.4. The leaf absorptance to solar radiation between 400 and 800 nm of *Encelia farinosa* with hairs intact and with hairs removed, and that of in *E. californica*. After Ehleringer and Björkman (1978).

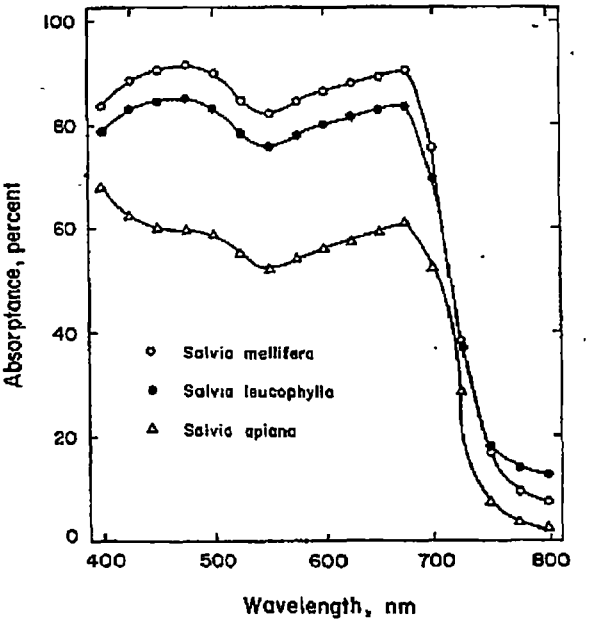


Figure2.5. Leaf absorptance value of solar radiation between 400 - 800 nm of three *Salvia* species growing along an aridity gradient. After Munz (1959, in Rodriguez *et al.* (1980)).

Based on these findings, it can be concluded that xeromorphic leaves tend to have pubescent surfaces. Such surfaces reflect solar radiation, thus reducing leaf temperature. The lower temperature reduces leaf transpiration. The pubescent surface also disturbs the gas diffusion across "the leaf-air interface" (Rodriguez *et al.*, 1983). It also functions as a water vapour saver, especially during windy weather.

Besides reducing leaf temperature, pubescence also produces another effect, that is reducing the absorption of photosynthetically active solar radiation, consequently it reduces photosynthesis rate (Figure 2.6) (Ehleringer and Mooney, 1978). It can be seen from the graph that *Encelia farinosa* plants differing in pubescence show different absorptance (a); it varies from 80, 65, 54 to 44% (400 to 700 nm). *Encelia californica* has the highest absorptance, that is 88%. The higher the leaf absorptance, the higher the net photosynthesis.

Several findings show different tendencies. Sharma *et al.* (1981) compared leaf cuticular and morphological variations in *Trifolium repens,* which grew in a humid, semi tropical and saline habitat, and in a terrestrial temperate, and less saline habitat. The results showed that the former trichome density (13,157 ± 62.3 per mm^2) was higher than that of the latter (5,921 ± 43.5). Rudall (1979) observed that some of the xeromorphic *Eriope* species have very few non glandular hairs. Another modification for coping with dry conditions occurred in *E. crassipes* subsp. *cristalinae.* This subspecies also had a thick cuticle, sometimes with cuticular striations and sometimes with a waxy surface.

Many researchers have carried out studies to investigate the relationship between leaf structural modification and habitat physical factors. Some of these have been attempts to demonstrate physiological aspects in support of structural alterations. Others tried to concentrate on quantitative studies on characters assumed to increase the boundary layer (Wood, 1923; Gausman and Cardenas, 1969; Ehleringer and Björkman, 1978) however, they were focused on individual structures and their results are often difficult to interpret across the whole leaf. Hill (1998) attempted to distinguish between characters increasing the boundary layer and scleromorphic characters by comparing them with fossil evidence, aimed to uncover the enigma of the dichotomy between xeromorphy and scleromorphy. This present research worked in the same area as Hill's, but it was conducted quantitatively to provide data to test Hill's hypothesis.

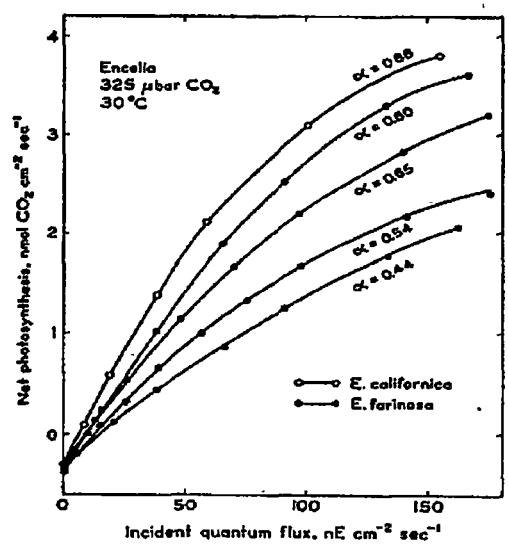


Figure2.6. Net photosynthesis versus incident quantum flux for a pubescent leaf of *Encelia farinosa* and for a glabrous leaf of *E. californica*. After Ehleringer and Mooney (1978).

A hypothesis, based on Hill's and Cowan's and other researchers' ideas, is proposed to limit the range of this research. Hill (1998) proposed that the presence of abundant trichomes, stomatal depressions, revolute leaf margins, and stomatal plugs, and also the combination of these characters increases the boundary layer. Cowan (1977) reported that 77% of total transpiration took place in the guard and subsidiary cells. Dickison (1975), Daghlian and Pearson (1977), Johnson (1980), and Carpenter (1994), reported that there was a positive correlation between the cuticle thickness and a dry environment. So far, it can thus be assumed that the more well developed the structures, the more protected the stomates.

2.2. Materials and methods

2.2.1. Sampling

Leaves of three genera of the Proteaceae, namely *Banksia, Grevillea* and *Orites,* were observed in this experiment. Due to low local availability of these plants in the field, and of their seeds from seed providers, and also the problems of seed dormancy and viability, they were sampled in slightly different ways. Glasshouse grown plants were used to assess which leaf characters were genetically fixed.

2.2.1.1. *Banksia*

Seeds of 23 species of *Banksia* (see Table 2.2) were germinated and grown in glasshouse conditions. Five leaves per species were taken from the tenth or eleventh nodes from the apex of seven month old seedlings (grown during autumn and winter 1996). They were then cut into small pieces taken from approximately the midlength of the leaves. Five pieces were preserved in formalin-acetic acid-alcohol (FAA) for anatomical examination (see resin embedding section procedure later). The remaining pieces were stored for morphological observation with scanning electron microscopy (SEM) (see SEM preparation procedure later) and cuticle analysis (see cuticle preparation procedure on later). Due to the varying and complicated structure of *Banksia* leaves, ten counts and measurements were applied to every single leaf piece.

Table 2.2. List of *Banksia* species sampled with habitat and distribution in Australia (After George, 1994). Rainfall figures are the ranges of the yearly mean.

Species	Habitat	Distribution
B. praemorsa (B1)	On coastal dunes, sometimes overlying granite or limestone, in heath. Annual rainfall 800 mm.	Western Australia, restricted to the south coast between Albany and Bald Island.
B. ashbyi (B2)	In deep red sand on dunes and plains, in shrubland. Annual rainfall 250-350 mm.	Western Australia, near the central west coast from North West Cape to Quabba and from Hamelin Pool to Mullewa and Coorow; also in the Kennedy Range east of Carnarvon.

Table 2.2. continued

Species	Habitat	Distribution
B. integrifolia var. *integrifolia* (B3)	Usually on coastal sand dunes and by inlets; often common. Annual rainfall 800-1200 mm.	South-eastern and eastern coastal Australia, from Wide Bay and Fraser Island, Queensland, to Port Philip Bay, Victoria.
B. sceptrum (B4)	In deep yellow or pale red sand in tall shrubland, often on dunes. Annual rainfall 300-400 mm.	Western Australia, near the central west coast between Hamelin Pool and Geraldton, extending inland almost to Mullewa.
B. attenuata (B5)	In deep sand, sometimes over limestone or laterite, in heath, shrubland and woodland. Annual rainfall 300-900 mm.	Western Australia, from Kalbarri to Leeuwin and east to Fitzgerald River; extends inland to Wongan Hills and Lake Grace.
B. conferta var. *conferta* (B6)	On steep rocky slopes (granite and sandstone) in scrub. Annual rainfall 1000-1500 mm	Queensland, on the Lamington Plateau and the Glasshouse Mountains.
B. caleyi (B7)	In white-grey sandy loam on plains, in mallee shrubland. Annual rainfall 550-600 mm.	Near the south coast of Western Australia, from South Stirling to the West River and inland to Pingrup.
B. occidentalis (B8)	In sand or peaty sand, usually on swamp margins in tall shrubland or paperbark woodland; sometimes in coastal seepages and on coastal dunes. Annual rainfall 350-800 mm.	Western Australia, along the south coast from Augusta to Cape Arid.
B. media (B9)	In sand, loam and clay, sometimes over limestone or granite, in heath, shrubland or open woodland. Annual rainfall 300-600 mm.	Southern Western Australia, from near the eastern end of the Stirling Range to Israelite Bay, with outlying populations at Point Culver and Toolinna; extends inland to Lake Cronin.
B. laevigata ssp. *laevigata* (B10)	In rocky soil (spongolite and laterite) in shrubland and open woodland. Annual rainfall 400- 500 mm.	Western Australia, along the lower Fitzgerald River and in the Ravensthorpe Range.

Table 2.2. continued

Species	Habitat	Distribution
B. baxteri (B11)	In deep white or grey sand on plains and dunes, in tall shrubland. Annual rainfall 500-700 mm.	Near the south coast of Western Australia, from the Stirling Range to the Oldfield River.
B. serrata (B12)	On consolidated coastal dunes, and in sand over sandstone on the coastal plain and in the Blue Mountains; usually in woodland, but when low in tall shrubland. Annual rainfall 800-1200 mm.	A common, near coastal species, extending from Cooloola, Queensland, south to Wilson's Promontory, Victoria, with an outlying population at Sisters Creek, north-western Tasmania.
B. seminuda (B13)	In red loam or gravelly loam along rivers and creeks in Jarrah-Marri forest; occasionally on sandy flats and coastal dunes. Annual rainfall 900-1300 mm.	Western Australia, from Banksiadale to Broke Inlet and east to Two Peoples Bay.
B. lemanniana (B14)	In rocky sand (quartzite) or rocky loam (laterite) on hillsides and plains, in tall shrubland or low woodland. Annual rainfall 500-600 mm.	Western Australia, near the south coast from West Mt Barren to Ravensthorpe and east to the rabbit proof fence.
B. baueri (B15)	In deep white or grey sand and in shallow sand over laterite or quartzite, in low shrubland and mallee shrubland. Annual rainfall 350-650 mm.	Western Australia, from Kweda and Toolibin to Bremer Bay and east to Munglinup; also on the South Stirling plains.
B. robur (B16)	In sand or peaty sand, usually swampy, in low woodland and in sedge-heathland. Annual rainfall 800-1100 mm.	Queensland and New South Wales; in Queensland between Cooktown and Mareeba, near Bowen, and between Shoalwater Bay and Coolangatta; in New South Wales between Kempsey and Wollongong.
B. verticillata (B17)	On or beside granite outcrops, usually exposed, sometimes in tall shrubland. Annual rainfall 800-1100 mm.	Western Australia, near the south coast at Walpole and from Albany to Two Peoples Bay.

Table 2.2. continued

Species	Habitat	Distribution
B. spinulosa var. *spinulosa* (B18)	In sand, loam and clay-loam, often over sandstone or granite, on flats and hillsides in open forest and woodland. Occurs both on the coastal plain and on adjacent ranges. Annual rainfall 600-1400 mm.	Queensland between Mossman and Ravenshoe and in the south-east from Coria Bay southwards, and New South Wales from the Colo and Hawkesbury Rivers south almost to the Victorian border.
B. brownii (B19)	In shallow sand over laterite, in woodland; in shale in gullies, in woodland; and among rocks on mountains, in heath. Annual rainfall 600-800 mm.	Western Australia, from Albany to the Stirling Range.
B. dryandroides (B20)	In clay-loam and sandy loam, sometimes over gravel, on low-lying flats, in shrubland and low woodland; also on sandstone hills in heath. Annual rainfall 700-800 mm.	Western Australia, near the south coast between Narrikup and Beaufort Inlet.
B. nutans var. *cernuella* (B21)	In deep white or grey sand, sometimes over gravel, in tall shrubland and open heath; sometimes on consolidated coastal dunes. Annual rainfall 350-500 mm.	Western Australia, near the south coast, from the Pallinup River to Israelite Bay.
B. ericifolia var. *ericifolia* (B22)	In deep sand, sandy loam, or sand over sandstone, in shrubland and woodland; sometimes in semi-swampy places. Annual rainfall 800-900 mm.	New South Wales, near the central coast and on adjacent ranges between Collaroy and Jervis Bay.
B. aemula (B23)	On consolidated sand dunes, in swales, on flats and sometimes on sandstone, in low woodland or tall shrubland (wallum). Annual rainfall 800- 1200 mm.	Queensland and New South Wales, from Bundaberg to Sydney, in near-coastal areas.

2.2.1.2. *Grevillea*

Leaves of 16 species of *Grevillea* (Table 2.3) were sampled from a private garden in Stawell, Victoria. It was presumed that these leaves represent a good sample of the leaf character range in *Grevillea* (it has more than 340 species - Olde and Marriot, 1994). Unlike *Banksia* leaves, which were harvested from juvenile and adult plants, the *Grevillea* leaves were harvested only from adults. Efforts to grow *Grevillea* from seed were not very successful, because of poor seed availability and/or because of poor seed viability and germination rates.

Five leaves per species were used as replicates. Every leaf was cut into small pieces taken from approximately the midlength of the leaf. Five pieces were fixed in FAA solution for anatomical examination (see later) 10 pieces were used for morphological observation (see SEM preparation procedure later) and cuticle analysis (see cuticle preparation procedure later).

Table 2.3. List of *Grevillea* species sampled with the habitat and distribution in Australia (Adapted from McGillivray, 1993 and Olde and Marriot, 1994). Rainfall figures are the ranges of the yearly mean.

Species	Habitat	Distribution
G. johnsonii (G1)	Rocky situations on sandstone. Rainfall 800-1000 mm.	New South Wales: restricted to the Goulburn River catchment in the area. *c.* Merriwa-Bylong-Nullo Mountain-Gungal, and the Capertee River catchment where it is known only from Mt Gundangaroo.
G. aquifolium (G2)	Heath and woodland, usually on sandy (occasionally calcareous in South Australia) soils; sometimes growing among sandstone rocks; sometimes in moist situations beside streams or in 'soak ' areas; occasionally growing on or near disturbed sites. Rainfall 375-1000 mm.	Victoria: from the Grampians area to Little Desert, and in the Portland district. South Australia: from *c.* 25 km north-east of Robe to the coast south-west of Mt Gambier.

Table 2.3. continued

Species	Habitat	Distribution
G. aspera (G3)	In heath, scrub, or occasionally woodland, often on rocky quartzite slopes, occasionally in clay loam or lateritic soils. Rainfall 200-220 mm.	South Australia; in various locations along more or less the length of the North Flinders Ranges; on the Eyre Peninsula including Mt Greenly, Marble Range, Port Lincoln area, Blue Range, Kimba area, and the Gawler Ranges, and ? Western Australia (Rawlinson Range).
G. sericea subsp. *sericea* (G4)	Usually in dry sclerophyll forest, shallow sandy soils on sandstone. Rainfall 500-1200 mm.	New South Wales-Central Coast from southern Sydney to near Newcastle; Blue Mountains area of the Central tablelands; Goulburn River catchment on the Central Western Slopes.
G. lavandulacea (G5)	In sandy or light-loam soils on a variety of acidic substrates, occasionally on limestone; usually in eucalypt woodland with a heathy understorey or in shrubland. Rainfall 200-700 mm.	Victoria , west of *c.* 144°E; south-east of South Australia-on the mainland south of 31°S and east of 138°E, also Kangaroo Island; one doubtful record from Yorke Peninsula.
G. spinosissima (G6)	In heath or shrubland on well-drained sandy to gravelly lateritic loams. Rainfall 325-400 mm.	Western Australia-western part of Avon Botanical District, from Wongan Hills area to about Quairaiding.
G. phanerophlebia (G7)	In open scrub or heath in sandy or gravelly loam soil. Rainfall 250-350 mm.	Western Australia: South-west Botanical Province: Irwin and possibly Avon districts, from Mingenew to near Mullewa.
G. pilosa ssp. *pilosa* (G8)	In heath, shrubland, or mallee scrub on gravelly soils. Rainfall 250-400 mm.	Western Australia: western half of Roe Botanical District in the area *c.* Nyabing-Lake King-Mt Holland (*c.* 32°09'S, 119°44'E), and Eyre district NW of Ravensthorpe.
G. lissopleura (G9)	In open shrubland on well-drained stony soils. Rainfall 300 mm	Western Australia-known only from one collection locality at *c.* 31°58' S, 119°39'E, near Mt Holland (Roe District).
G. nudiflora (G10)	In tall shrubland, mallee, or heath, in sandy to loamy soils over laterite, quartzite, shale. Rainfall 500-800 mm.	Western Australia-South-west Botanical Province: Eyre and Roe Districts and far SE corner of Darling District; from *c.* Mt Barker to Cape Arid area.

Table 2.3. continued

Species	Habitat	Distribution
G. bipinnatifida (G11)	Wandoo (*Eucalyptus wandoo*), Jarrah (*E. marginata*), or Marri (*E. calophylla*) woodland; usually on lateritic loamy soils, occasionally recorded from granitic soils (*Seabrook 431*, Helena Valley). Rainfall 600-1000 mm.	Western Australia: from Babilion Range to the Collie district usually near the scarp of the Darling Range; in the northern part of its range it appears to be restricted to Babilion Range (near Mogumber) and Red Hill (near Bindoon).
G. beadleana (G12)	Among granite outcrops with pockets of gritty-loamy soil and in patches of scrub and forest. Rainfall 800-1250 mm.	About 70 km W of Tenterfield on the Mole River catchment; c. 4 km SSW. of Chaelundi Falls, Guy Fawkes River National Park, 30°05' S, 152°19' E; and Walcha.
G. iaspicula (G13)	On limestone; survives in rocky outcrop areas only. Rainfall 600-800 mm.	New South Wales-Wee Jasper area *c.* 50 km north-west of Canberra.
G. manglesii ssp. *dissectifolia* (G14)	Moist woodland situations. Rainfall 850-1000 mm.	Western Australia: South-west Botanical Province-northern part of Darling District, from Avon River area to about Narrogin; in the area Jarrahdale-Dwelingup-North Bannister.
G. caleyi (G15)	Restricted to woodland or shrubland on lateritised sandstone ridgetops. Rainfall 1000-1200 mm.	In the Mona Vale-Terrey Hills-Middle Harbour area to the north of Sydney, roughly in the vicinity of 33°40'S, 151°15' E.
G. willisii (G16)	Rocky situations near streams, on granite. Rainfall 800-1100 mm.	Eastern Victoria. *G. willisii* ssp. *willisii* occurs on the catchments of the Mitta Mitta River and Nariel Creek, parts of the upper Murray River system. *G. willisii* ssp. *pachylostyla* occurs in the vicinity of the southern-flowing Buchan River and its tributaries, part of the Snowy River system.

2.2.1.3. *Orites*

Leaves of 6 *Orites* species (Table 2.4) were sampled from glasshouse grown plants at the Plant Science Department, University of Tasmania (4 Tasmanian species; Wrigley and Fagg, 1991) and from The National Botanical Gardens, Canberra (2 of the mainland species; Wrigley and Fagg, 1991). Because the mainland species were adapted to an unnatural habitat, the Tasmanian species were also grown in such a habitat (in a glasshouse).

Every *Orites* leaf was cut into small pieces at approximately the midlength of the leaf in the same way as the *Banksia* and *Grevillea* leaves, and treated in three different ways, in order to observe the morphological and anatomical structures. Ten counts and measurements were conducted for every leaf piece, due to structural variety.

Table 2.4. List of *Orites* species sampled with the habitat and distribution in Australia (After Wrigley and Fagg, 1991). Rainfall figures are the ranges of the yearly mean.

Species	Distribution and ecology
O. acicularis (O1)	A common plant endemic to mountain plateaus of Tasmania at elevations above 1000 m. Habitat annual rainfall 1018-2983 mm.
O. revoluta (O2)	A common plant endemic to mountain plateaus in Tasmania. Habitat annual rainfall 842-2819 mm.
O. excelsa (O3)	Grows in warm temperate and subtropical rainforests, mostly at altitudes above 600 m. There is an outlier population in north Queensland. Habitat annual rainfall 716-7095 mm.
O. diversifolia (O4)	Occurs at low altitudes in temperate rainforest and on mountains at over 1000 m. Endemic to Tasmania. Habitat annual rainfall 750-3292 mm.
O. lancifolia (O5)	Common in alpine heaths and woodlands in mainland southeastern Australia, often among rocks. Habitat annual rainfall 1273-2452 mm.
O. milliganii (O6)	Grows in alpine heaths at altitudes above 1000 m. Endemic to Tasmania. Habitat annual rainfall 1094-3325 mm.

2.2.2. Resin embedding section

This process consists of 6 steps, namely fixation, dehydration, infiltration, polymerisation, cutting and mounting, and section staining (Agar Scientific Ltd., undated). Specimens (leaf pieces) should be fixed in FAA for at least 2 hours (Blake, pers. comm., 1996). All samples used in this experiment were fixed for 7 days, due to the hardness of their structures. After fixation they were transferred into a graded alcohol series for dehydration, at first two changes in 70% alcohol for at least 30 minutes each and then two changes in absolute alcohol for at least 30 minutes each. For harder specimens, longer dehydration (overnight) was required in order to remove water completely.

The next step was very dependent on the success of the dehydration process. If the samples were not dehydrated properly, they could not be well infiltrated by resin. Sometimes an extra step, transferring samples to a solution of 50% volume of absolute alcohol and 50% volume of resin was required, especially for hard specimens (Blake, pers. comm., 1996). Well infiltrated samples were shown by translucence; they also sank to the bottom of the container (embedding capsule). Unsuccessful infiltration could be repeated by reversing the procedure.

The well infiltrated samples were then transferred to medium size flat bottomed embedding capsules, one sample each. The embedding capsules were filled with resin, and the lids were closed tightly. The next step was polymerisation; the infiltrated samples were polymerised at 60°C in an oven, for 20 to 24 hours. This process could be done in cold (room temperature) conditions as well (Agar Scientific Ltd., undated); however this was not done in this experiment.

The polymerised resin was quite hard and the specimen had to be oriented in a correct position; that was with its cross section facing the microtome blade. A hacksaw was used to separate the sample from the excess of polymerised resin. The resin was removed from the sample as much as possible to avoid wrinkles in the resin, when it was cut and mounted on a slide. Afterwards, the sample was glued on a flat surface of resin in the correct position, with resin glue and left to dry for about 5 hours.

When the specimen was fixed enough, it was sectioned using a Heidelberg Rotary Microtome (HM 340 E), which was equipped with a glass knife made using an LKB Knife Maker (Type 7801 B). The sections were about 3 μm thick, and were put on a slide coated with Poly-L-Lysine (Sigma-P1524 hydrobromide) solution. In order to flatten wrinkled sections, a drop of 30 - 40% acetone was applied to float and spread the sections out (Agar Scientific Ltd., undated). Next, they were left to dry on a hot plate at 60 - 70°C for about 5 minutes.

The section was then stained with safranin for about 30 seconds, washed with running water gently, left to dry on a hot plate at 60 - 70°C for about 2 minutes. A drop of DPX was used to mount a cover glass on the section.

2.2.3. Cuticle preparation for transmitted light microscopy

Leaf pieces were put in a vial and soaked in a 10% aqueous hydrogen peroxide solution to which was added a teaspoon tip of tetra-sodium pyrophosphate crystals. The vial was put on a hot plate at low temperature (70°C) for several hours or left overnight, depending on the hardness of the samples. When the cuticles were ready, they were carefully washed with cold water, stained with safranin and mounted with glycerine jelly. In order to get clean cuticles, before they were stained, leaf cell remainders were brushed away gently.

2.2.4. SEM preparation for morphological observation

Fresh or dry specimens (leaf pieces) were fixed on an aluminium stub using double- sided adhesive tape. The specimens were then sputter coated with a 25 - 30 nm thickness of gold using a Balzers Union sputtering device. Afterwards, the gold- coated specimens were examined using an Environmental Scanning Electron Microscope in hivac mode and their surface images were recorded on Ilford FP4 film. Every specimen was examined for both stomate and hair position on the abaxial surface (most of the specimens

except *Orites acicularis* and some *Grevillea* species have this type of distribution).

2.2.5. Data collection

Anatomical characters that could be interpreted as providing stomatal protection could not always be measured quantitatively, and so a qualitative measure was applied. The characters were:

- leaf shapes and margins;
- the presence of hair clumps; and
- the presence of cuticle projections

2.2.5.1. *Banksia*

Due to the variety and complexity of *Banksia* leaf structure, characters measured in this genus were slightly different from those measured in *Grevillea* and *Orites*. Those characters, which were difficult to quantify, were measured qualitatively. There were four quantitative characters; depression index (Id), index of recurved margin (Irm), hair index (Ih) and epidermis index (Ie) and three characters measured; the thickness of stomatal, upper epidermis and lower epidermis cuticles. The details of measurements, counts and calculations are as follows.

1. Depression

Characters measured are shown diagrammatically in Fig. 2.7.

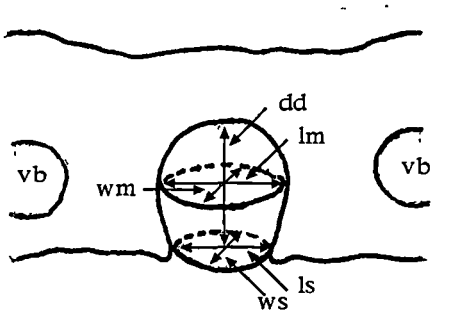


Figure 2.7. Diagram of a cross section of a *Banksia* leaf.

dd = depression depth; ls = length of surface plate; ws = width of surface plate; wm = width of median plate; lm = length of median plate; vb = vascular bundle.

The deeper stomate positions were assumed to be more protected than the shallower positions and the smaller surface plates of depression tended to be more protected than the larger ones. These assumptions were outlined in formulating the depression index. Moreover, some approaches to eliminate the effects of the depression nature and shape were the main considerations.

The depression index in *Banksia* was formulated as follows.

$$Id = \frac{dd^2}{as} + \frac{am - as}{as}$$

where, as = area of surface plate and am = area of median plate. These areas were assumed to have an elliptical shape, and thus were calculated as $\pi(1/2$ ws x $1/2$ ls) and $\pi(1/2$ wm x $1/2$ lm) respectively.

Therefore,

$$Id = \frac{dd^2}{\pi/4(ws \times ls)} + \frac{(wm \times lm) - (ws \times ls)}{ws \times ls}$$

$$= \frac{4\,dd^2}{\pi(ws \times ls)} + \frac{wm \times lm}{ws \times ls} - 1$$

$$= \frac{4\,dd^2 + \pi(wm \times lm)}{\pi(ws \times ls)} - 1$$

For simplicity the constant values were not included in the calculation
So that,

$$Id = \frac{dd^2 + (wm \times lm)}{(ws \times ls)}$$

The higher the depression index value, the more protected the stomate is.

2. Recurved margin

Characters measured are shown diagrammatically in Fig. 2.8.

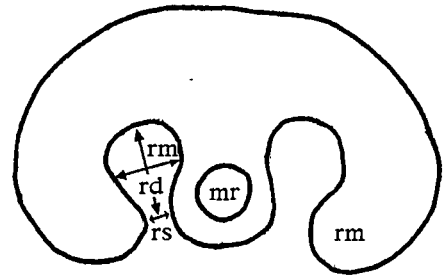


Figure 2.8. Diagram of a cross section of a recurved leaf. rs = the width of the surface plate; rm = the width of median plate; rd = recurved depth; rm = recurved margin; mr = midrib.

Recurved margins are defined as those which bend backwardly or downwardly (Debenham, undated). Some *Banksia* leaves do not show any depression on their surfaces, they possess recurved leaf margins instead; or they show both characters. It is assumed that recurved margins have a similar function to depressions, that is, to keep stomates protected from the environment. Hence, index of recurved margin (Irm) was determined as follows.

$$Irm = \frac{rd}{rs} + \frac{rm - rs}{rs}$$

$$= \frac{rd}{rs} + \frac{rm}{rs} - 1$$

$$= \frac{rd + rm}{rs} - 1$$

For simplicity the constant values were not included in the calculation
So that,

$$Irm = \frac{rd + rm}{rs}$$

3. Epidermis

Fig. 2.9 shows diagrammatically a typical *Banksia* leaf surface.

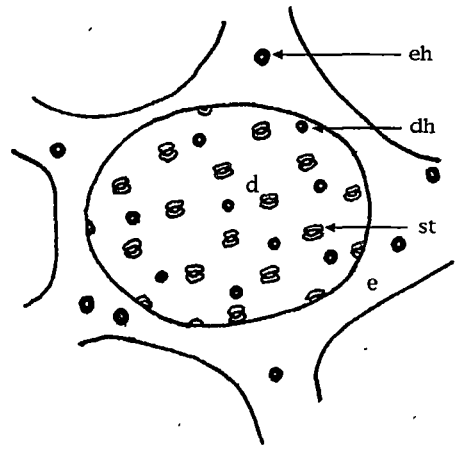


Figure2.9. Diagram showing the distribution of stomates and hairs in a depression and on the epidermis.
e = epidermis; eh = epidermis hairs; d = depression; s = stomates; dh = depression hairs

More hairs surrounding stomates may provide more protection to the stomates (Figure 2.9); hence the epidermis index (Ie) was formulated as follows.

$$Ie = \frac{ddh + deh}{ds}$$

dd = density of depression hairs/μm^2.
de = density of epidermis hairs/μm^2.
ds = density of the stomates/μm^2.

4. Hairs

Although there is a tendency for long hairs to be curly (Fig. 2.10), hair length and thickness were assumed to have the same roles in forming the boundary layers. The hair index (Ih), therefore was formulated as follows.

$$Ih = (ddh \times ldh) \times dd + (deh \times leh) \times de$$

ddh	=diameter of depression hairs
ldh	=length of depression hairs
dd	=density of depression hairs
deh	=diameter of epidermis hairs
leh	=length of epidermis hairs
de	=density of epidermis hairs

The higher the value of the hair index, the more protected the stomate is.

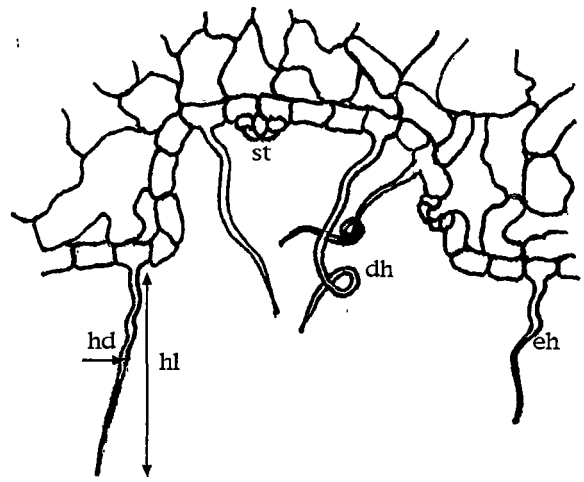


Figure2.10. Diagram of a depression showing stomates and hairs either in the depression or on the epidermis. hd = hair diameter (at about a half length); hl = hair length; dh = depression hair; eh = epidermis hair ; st = stomate

Because it was assumed that all cuticles on a leaf have direct or indirect roles in protecting excessive transpiration, their thickness was measured (Figure 2.11). Three cuticle measurements, stomatal cuticle thickness (sc), the thickness of the upper epidermis cuticle (uec) and of the lower epidermis cuticle (lec) were recorded.

5. Cuticle

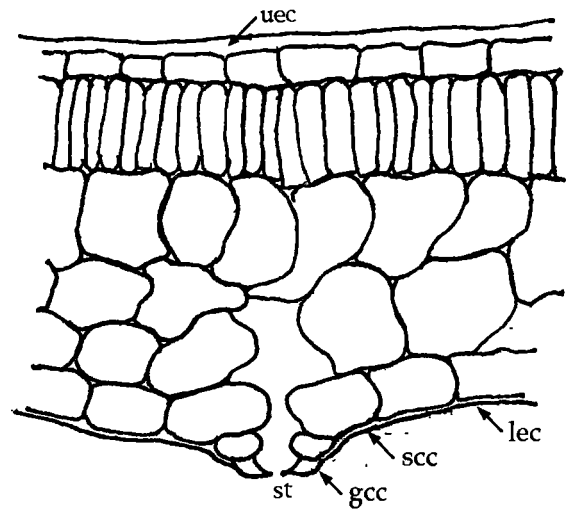


Figure2.11. Diagram of a stomate in cross section. gcc = the thickness of the guard cell cuticle; scc = subsidiary cell cuticle; uec and lec = upper and lower epidermis cuticle; st = stomate.

Assuming that the characters formulated above provided protection to stomates altogether by increasing the boundary layers, the stomatal protection component was formulated as follows.

Stomatal protection component = Index component + Cuticle component

where,

Index component = Depression index + Recurved margin index + Hair index + Epidermis index

= Id + Irm + Ih + Ie

and

Cuticle component = the thickness of stomatal cuticle + the thickness of the upper epidermis cuticle + the thickness of the lower epidermis cuticle

= Sc + Uec + Lec

Therefore,

Spc = Id + Irm + Ih + Ie + Sc + Uec + Lec

This means that the higher the value of the stomatal protection component, the stronger the boundary layer formed. However, due to the unit measurement differences between index and cuticle components, it is not possible to represent Spc as a numerical value. Moreover, in order to avoid biased results about the value of every component, each component was separately analysed.

2.2.5.1. *Grevillea* and *Orites*

Unlike *Banksia,* stomatal depressions in *Grevillea* and *Orites* protect single stomates. Therefore, the measurement of the depression was slightly different from those for *Banksia.* Furthermore, the hairs in *Grevillea* and *Orites* were also different in respect to their positions and/or shapes. In these genera, they were only found on the epidermis, thus the formula designed for determining the hair index in *Banksia* was slightly modified. Cuticle and epidermis index were applied in the same way as in *Banksia*. The details of measurements, counts and calculations are as follows.

1. Depression

Characters measured are shown diagrammatically in Fig. 2.12.

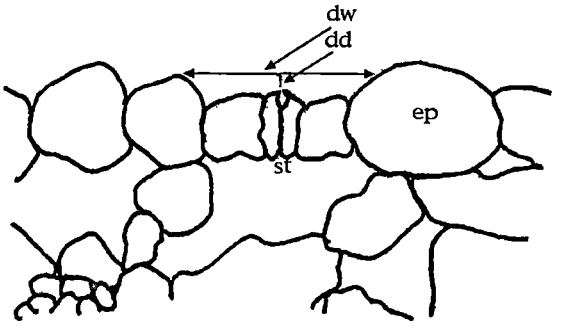


Figure 2.12. Diagram of a stomatal depression found in *Grevillea* and *Orites*. dd = depression depth; dw = depression width

Depression index (Id) was determined in a simpler way than in *Banksia*. In *Grevillea* and *Orites*, every single stomate lay in an epidermis depression or was protected by cuticle projections. Special characters in a stomatal depression were considered as qualitative data, supporting the quantitative data, which were formulated as:

$$Id = \frac{dd}{dw}$$

2. Recurved margin

Characters for calculating the index of recurved margin (Irm) were the same as for *Banksia*. Special characters, which were unlikely to be measured quantitatively, were measured qualitatively as supporting data.

3. Epidermis

The epidermis in *Grevillea* and *Orites* leaves is simpler than in *Banksia*, because stomates and hairs lie on a flat surface. Therefore, the epidermis index (Ie) in *Grevillea* and *Orites* was formulated in a simpler way than that for *Banksia*.

$$Ie = \frac{dh}{ds}$$

dh = hair density
ds = stomate density

4. Hairs

In *Orites* and *Grevillea* epidermis hairs were the only hairs protecting stomates from excessive transpiration. *Grevillea* leaves have T-shaped hairs, whilst *Orites* have needle-shaped hairs. The hair index (Ih) for *Orites* was formulated as follows.

$$Ih = (hd \times hl) \times dh$$

hd = hair diameter
hl = hair length
dh = hair density per μm^2

The T-shaped hairs in *Grevillea* have two parts, horizontal and vertical. Horizontal parts were usually much longer than the vertical parts. Thus it was assumed that the former gave more protection than the latter. The hair diameters were also considered to have a role in stomatal protection. The bigger the hairs the more protective they were. Based on this assumption the hair index in *Grevillea* was formulated as follows.

$$Ih = [(dhp \times lhp) + (dvp \times lvp)] \times dh$$

dhp = diameter of horizontal part
lhp = length of horizontal part
dvp = diameter of vertical part
lvp = length of vertical part
dh = hair density per μm^2

5. Cuticle

As there was no morphological difference between the cuticle of *Grevillea* and *Orites* and that of *Banksia,* cuticle thickness was measured in the same way as for *Banksia.* The stomatal protection number in *Grevillea* and *Orites* was calculated as:

$$Spn = Id + Irm + Ih + Ie + Sc + Uec + Lec$$

2.2.6. Data analysis

Data were analysed using an analysis of variance with five replicates per species, in order to investigate whether there were differences in characters determined

among species observed. Every index calculated (Id, Irm, Ih and Ie) and the cuticle component (Sc, Uec and Lec) was analyzed separately. Species observed were then classified according to the value of every index calculated and cuticle thickness. Therefore, variation of the characters that were assumed to increase the boundary layer, could be investigated. In order to determine the intensity of the association between characters observed and the minimum annual rainfall, the maximum annual temperature, the maximum daily radiation and the maximum daily wind run, an analysis of correlation was applied.

2.3. Results

2.3.1. *Banksia*

A. Leaf Morphology

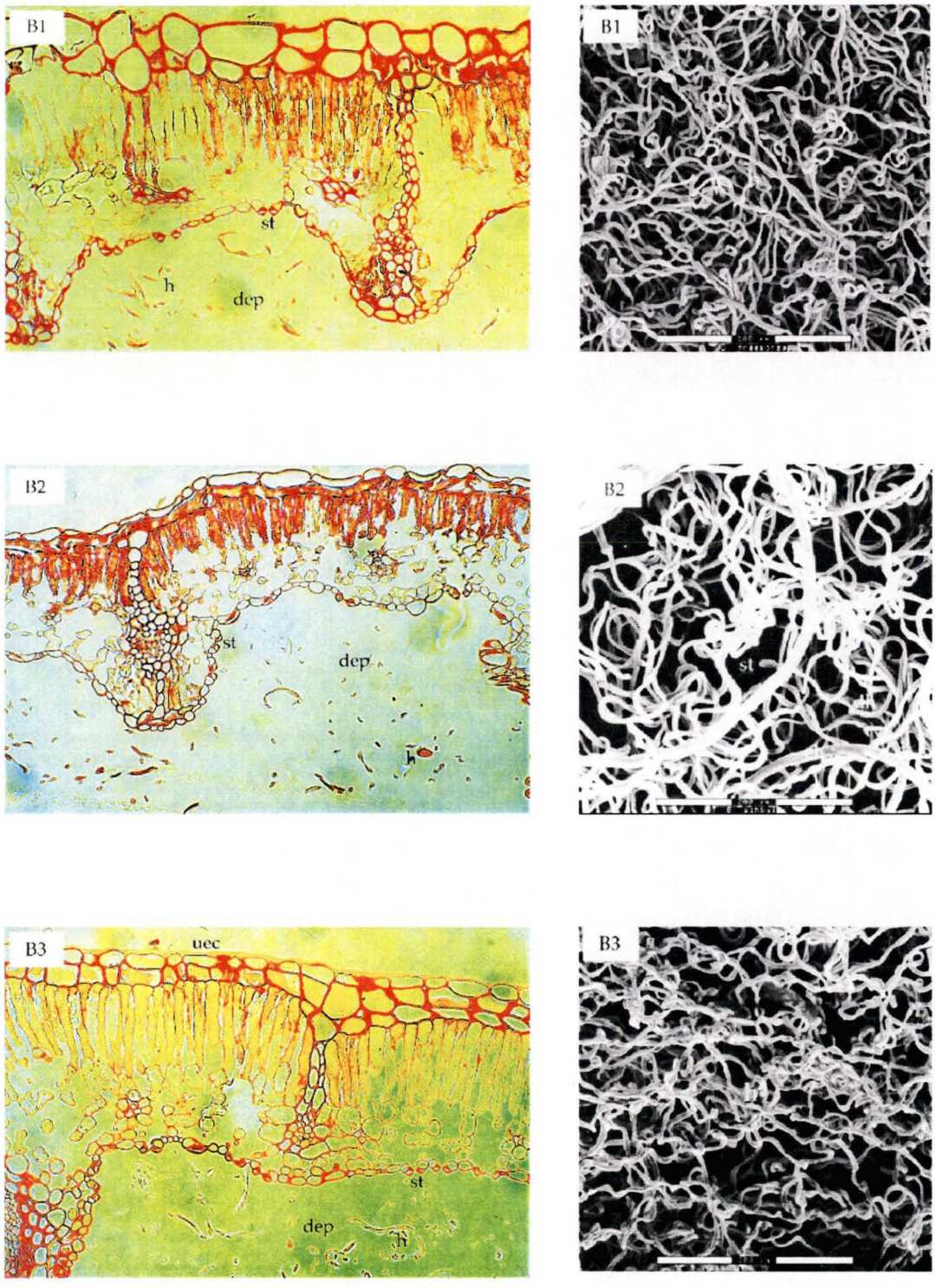
Banksia leaves are interesting morphologically due to the diversity of their shapes and sizes. Moreover, anatomically the diversity is more interesting since the stomatal positions range from exposed (*e.g. B. verticillata*) to very hidden (*e.g. B. blechnifolia*). It is assumed that this structural diversity represents plant response to climatic habitat (dryness) (Hill, 1994). This research tried to uncover more about this phenomenon.

Hair presence and density are also suspected to be an adaptation to dryness. *Banksia* also shows a range in hair density from low (*B. sceptrum*) to very dense (*B. nutans* var. *cernuella*). The very dense hairs are usually formed by curled hairs. Some species have very recurved margins with stomates protected inside. Species with dense hairs tend to have thinner lower epidermal cuticles and vice versa. Upper epidermal cuticles also tend to be thicker than the lower ones. Stomatal pits show various depth and widths among species observed. The adaptive significance of every character discussed above will be outlined in the following discussion.

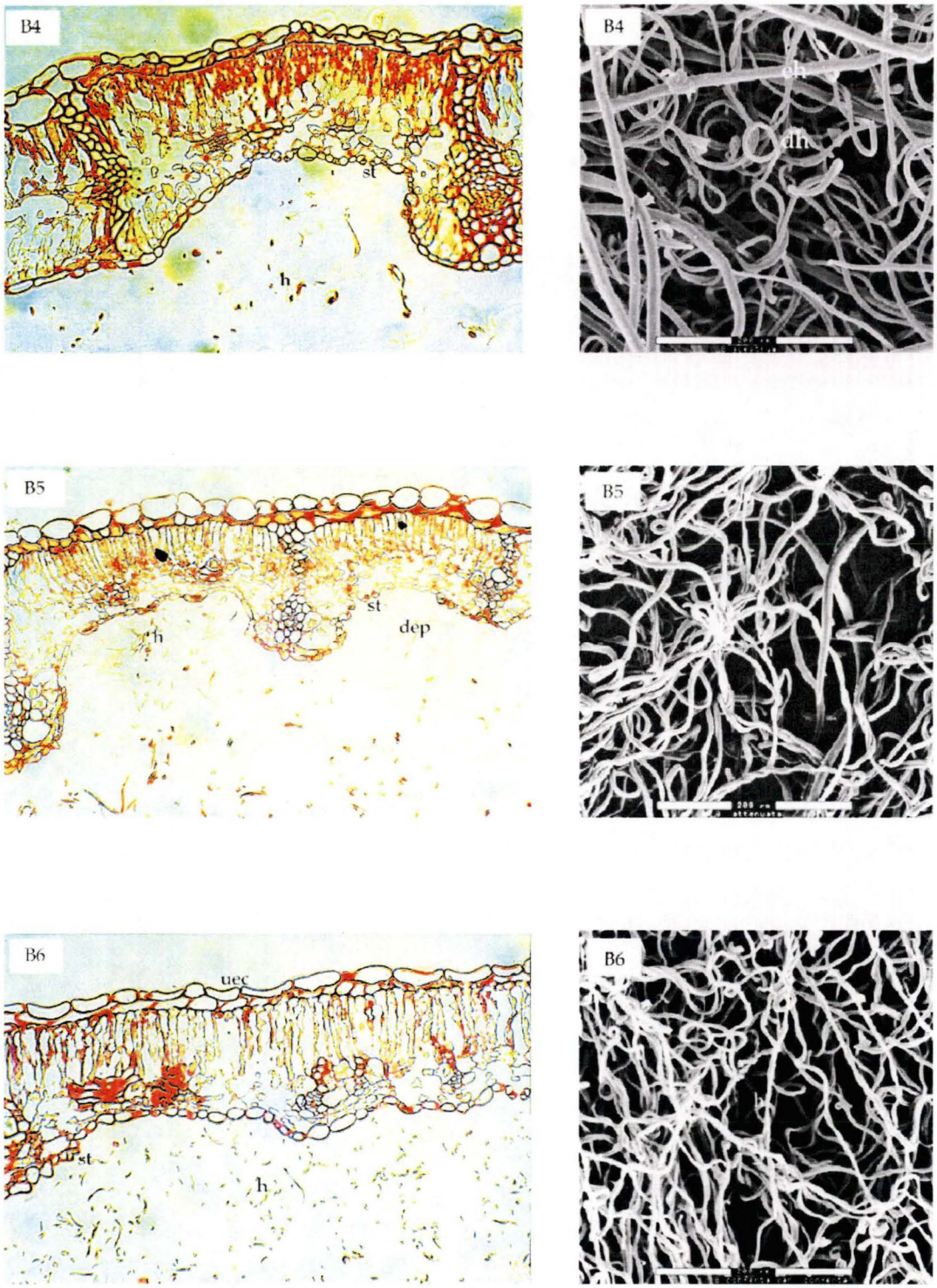
Morphological studies were conducted by observing the cross sections of leaves and Environmental Scanning Electron Microscope (ESEM) images of the lower surface of leaves of *Banksia* species. The following figures describe details of the character states observed in every species.

B. Correlations

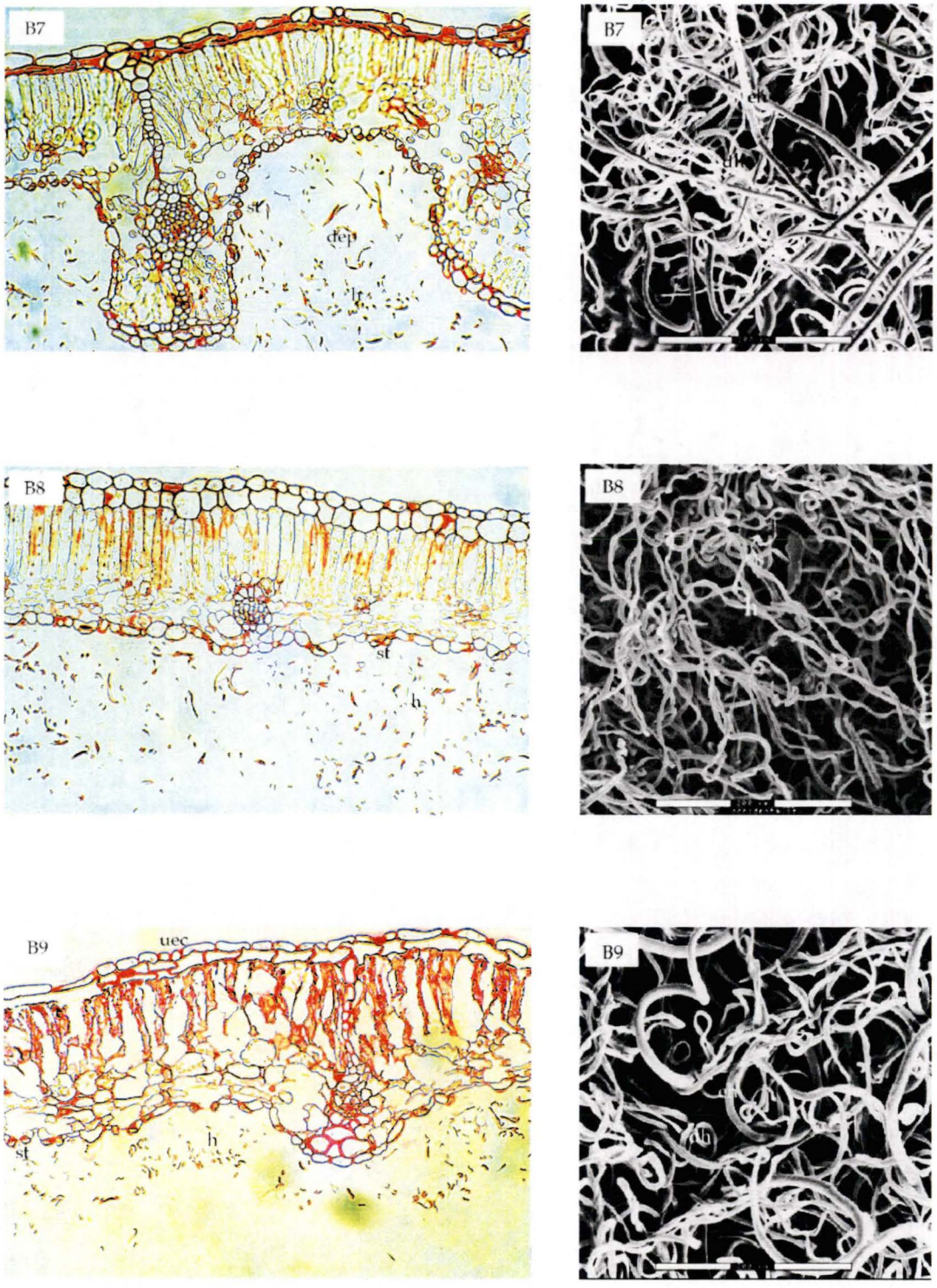
A correlation between components of stomatal protection and minimum of the range of annual rainfall was investigated for the purpose of predicting any tendency for a direct correlation. The lack of other climatic data for every species observed, however, was a barrier to understanding more about plant and habitat correlations. An attempt to solve the problem was made by designing a correlation model using estimated climatic factors, which were generated from herbarium locality data with ESOCLIM analysis (Hutchinson, 1991), and then correlated against the estimated climatic factors already calculated. The results are summarised in figs 2.13-2.18 and tables 2.5-2.7.



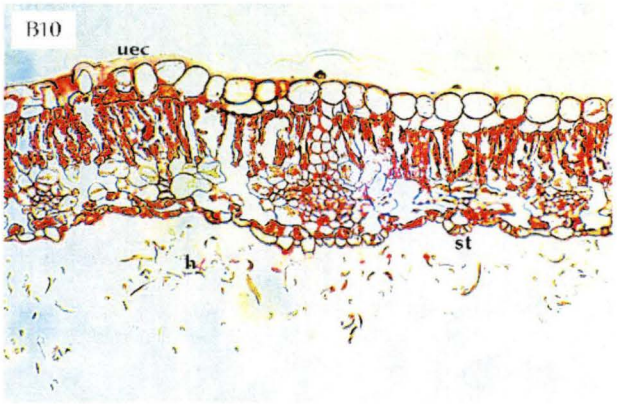
Figures 2.13a. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *Banksia praemorsa* (B1-left, 160x), *B. ashbyi* (B2-left, 160x), and *B. integrifolia* var. *integrifolia* (B3-left, 160x). The first two species possess depressions with abundant hairs and stomates in them. Moreover, *B. ashbyi* has two different types of hairs, namely depression and epidermis hairs. The last species does not have any depression, but abundant hairs cover the stomates.
dep = depression; dh = depression hairs; eh = epidermis hairs; h = hairs; st = stomates; uec = upper epidermis cuticle.

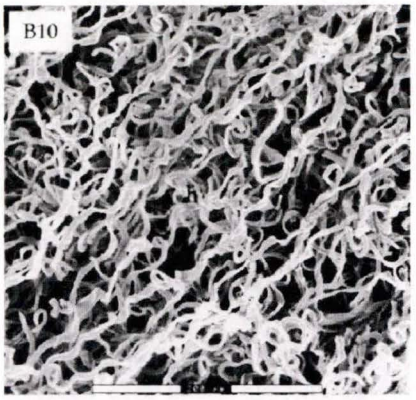


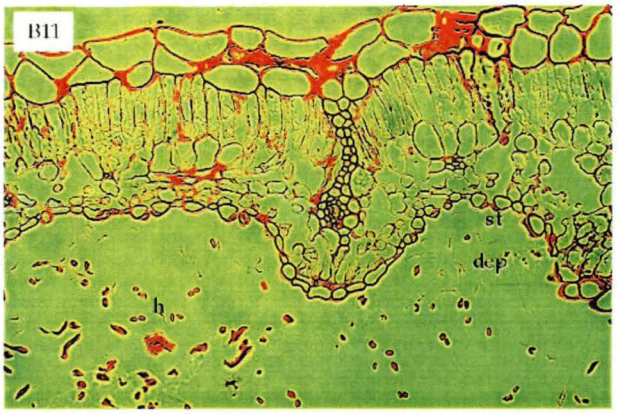
Figures 2.13b. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *B. sceptrum* (B4-left,160x), *B. attenuata* (B5-left, 160x), and *B. conferta* var. *conferta* (B6-left, 160x). The first two species have depressions and together with depression and epidermis hairs they protect stomates from excessive drying. The last species does not possess any depression, stomates are exposed on the leaf surface and protected by fine and dense hairs.
dep = depression; h = hairs; dh = depression hair; eh = epidermis hair; st = stomate; uec = upper epidermis cuticle.

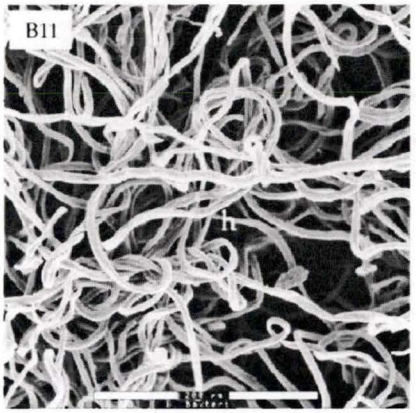


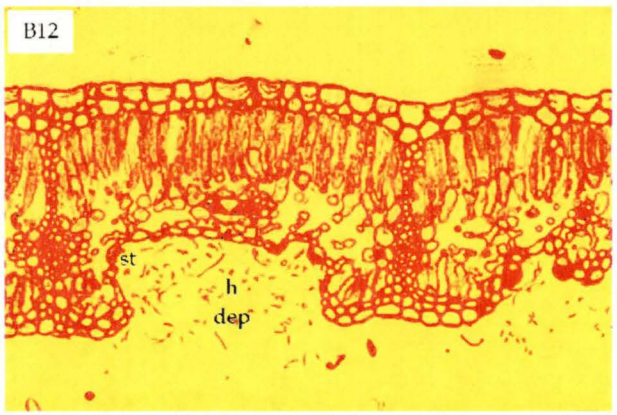
Figures 2.13c. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *B. caleyi* (B7-left, 160x), *B. occidentalis* (B8-left, 160x) and *B. media* (B9-left, 160x). *B. caleyi* possesses deep depressions with abundant stomates inside. *B. occidentalis* has stomates exposed on the leaf surface, however, dense hairs provide protection to the stomates. *B. media* has the stomates in depressions and also covers them with hairs.
dep = depression; dh = depression hairs; eh = epidermis hairs; h = hairs; st = stomate; uec = upper epidermis cuticle.

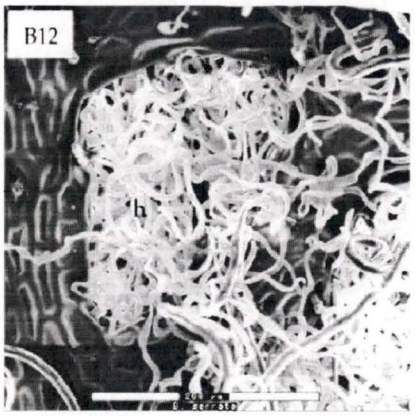




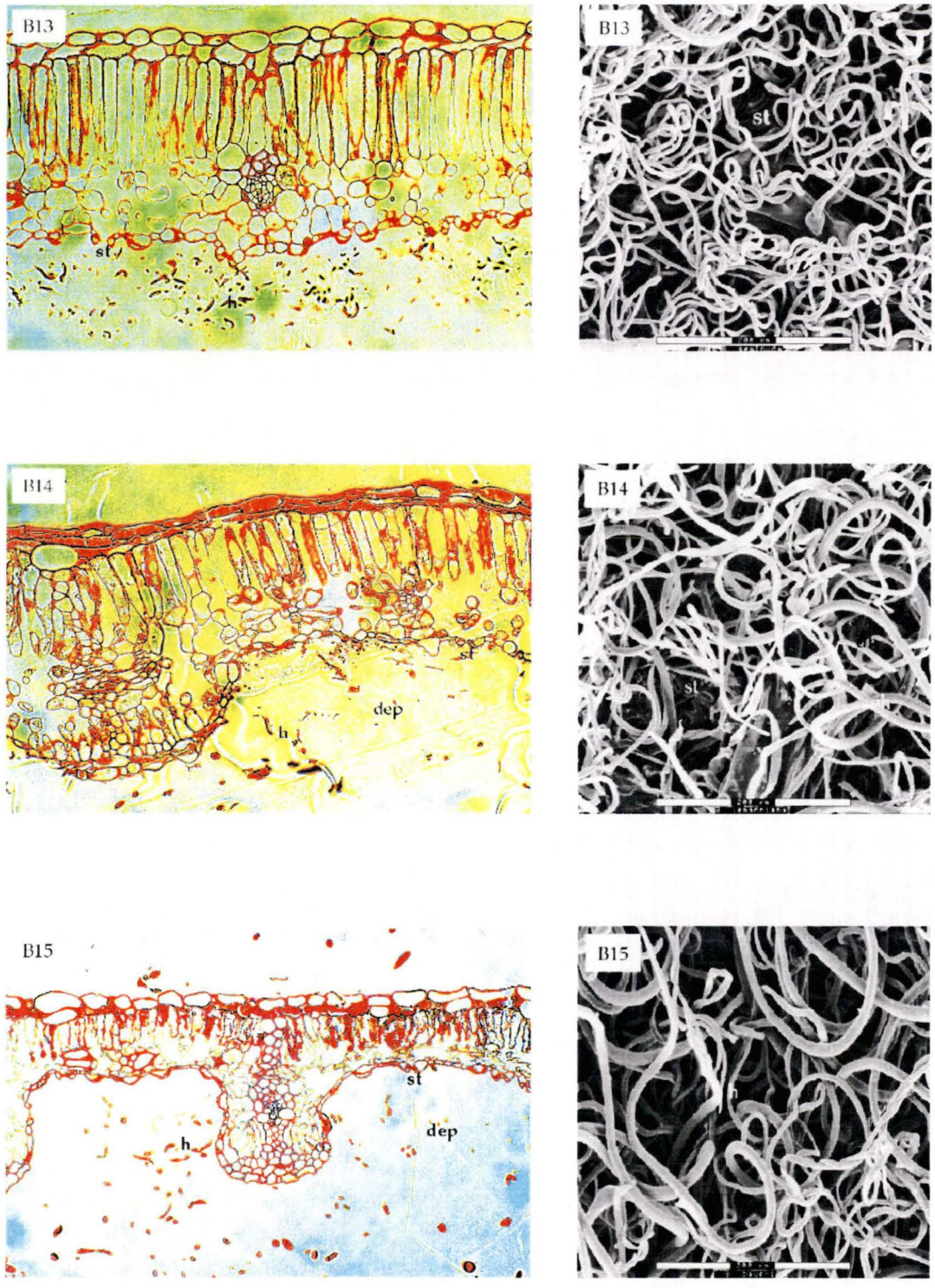




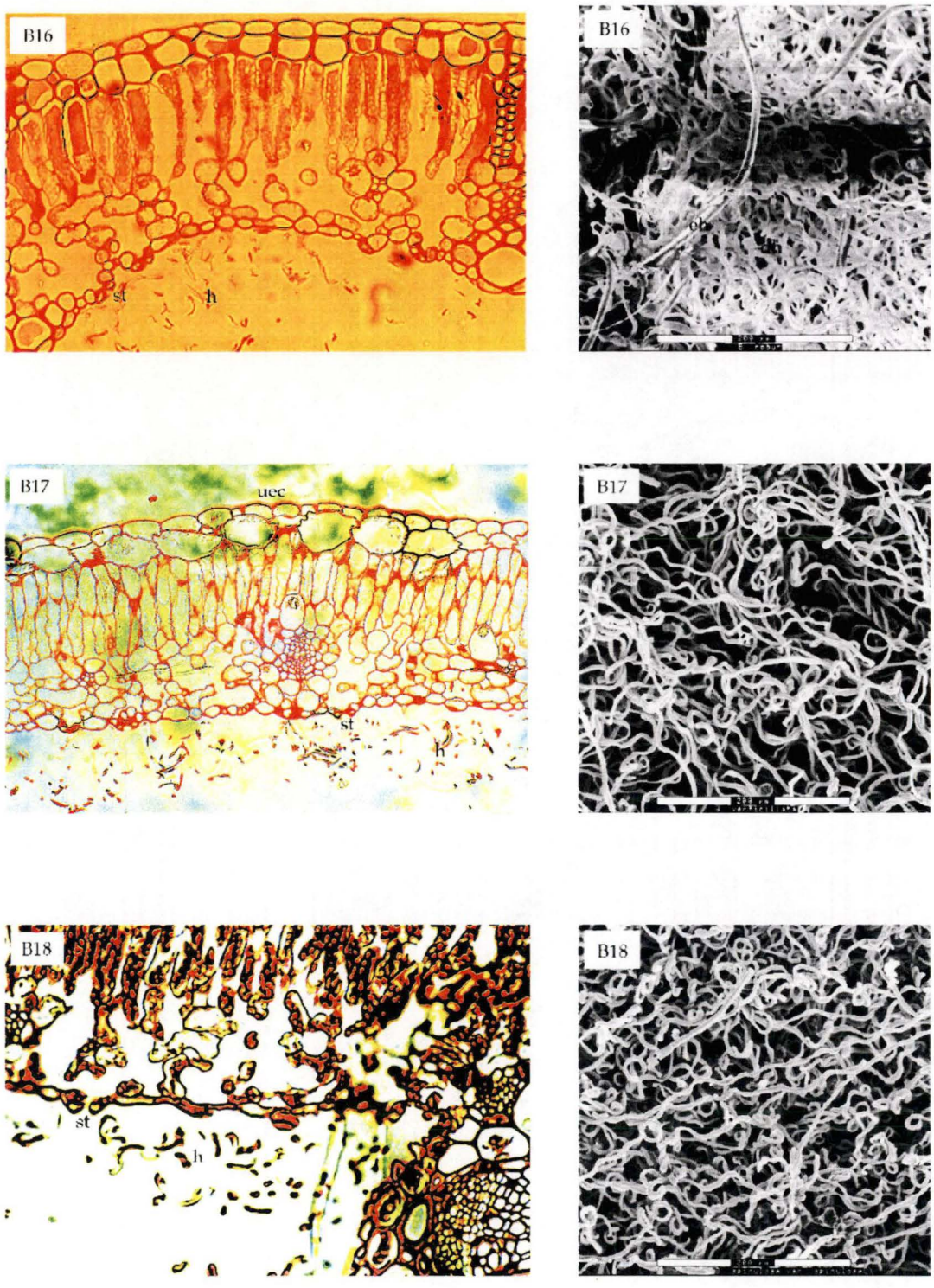




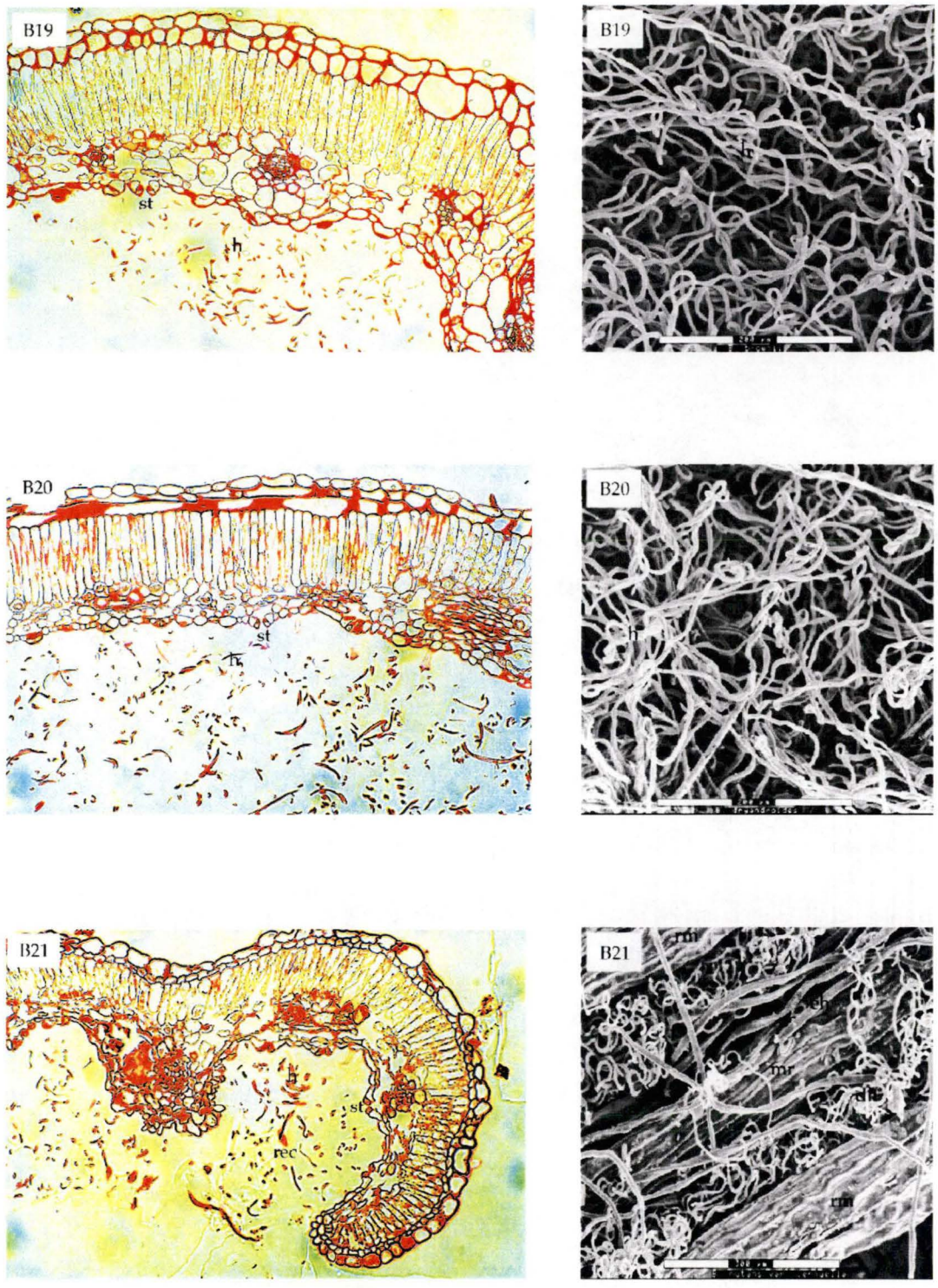
Figures 2.13d. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *B. laevigata* ssp. *laevigata* (B10-left, 160x), *B. baxteri* (B11-left, 160x), and *B. serrata* (B12-left, 160x). The first species has fine and dense hairs protecting the stomates which are exposed on the leaf surface. The last two species have depressions and with hairs confined to them, however in *B. serrata* hairs mostly occupy the depressions.
dep = depression; h = hairs; st = stomate; uec = upper epidermis cuticle.



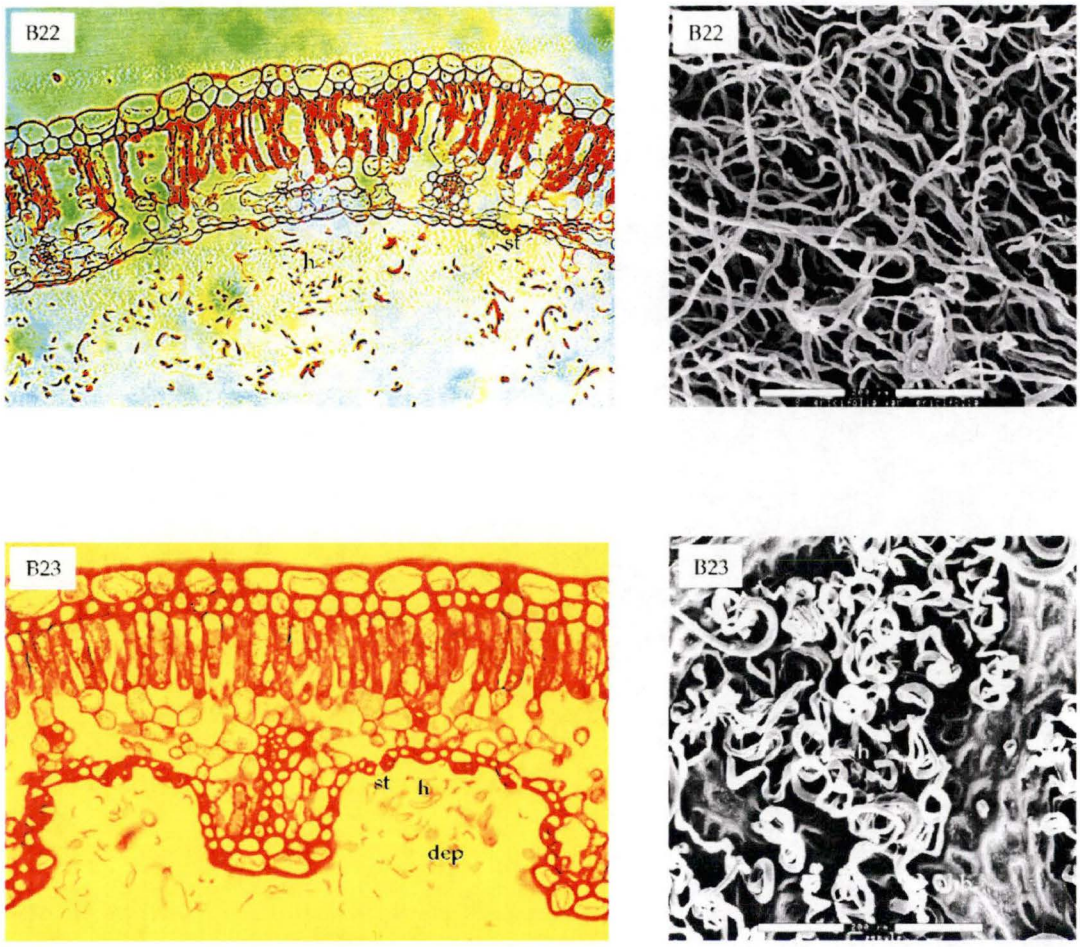
Figures 2.13e. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *B. seminuda* (B13-left, 160x), *B. lemanniana* (B14-left, 160x), and *B. baueri* (B15-left, 160x). The first species does not have any depression, seemingly hairs are the only structure covering the stomates. In the last two species some depressions are found and two different types of hairs *i.e.* depression and epidermis hairs. In these species the epidermis hairs seem to play a role in covering the stomates.
dep = depression; dh = depression hairs; eh = epidermis hairs; h = hairs; st = stomates.



Figures 2.13f. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *B. robur* (B.16-left, 160x), *B. verticillata* (B17-left, 160x), and *B. spinulosa* var. *spinulosa* (B18-left, 320x). The last two species do not have depressions or recurvation. However, both possess very dense hairs which very possibly give good protection to the stomates. The first species has depressions with stomates hidden inside, less hairs and no recurvation.
dep = depression; h = hairs; st = stomate; uec = upper epidermis cuticle



Figures2.13g. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *B. brownii* (B19-left, 160x), *B. dryandroides* (B20-left, 160x), and *B. nutans* var. *cernuella* (B21-left, 160x). These three species do not possess any depression. Their stomates are on the leaf surface, yet abundant hairs, especially in *B. brownii* and *B. nutans* var *cernuella*, provide good shelter for the stomates. Moreover, the presence of recurvation in the last two species, might provide protection to the stomates.
dep = depression; h = hairs; mr = midrib; rec = recurvation; rm = recurved margin; st = stomates.



Figures 2.13h. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *B. ericifolia* var. *ericifolia* (B22-left, 160X), and *B. aemula* (B23-left, 160X). The first species does not possess any depression, but its very dense hairs provide good shelter for the stomates. The second species has deep depressions, with curly hairs confined to them.
dep = depression; dh = depression hairs; h = hairs; st = stomates.

1. Stomatal protection components of *Banksia* and Rf correlation.

Table 2.5. Index values and cuticle thickness (mean ± s.e.) and habitat rainfall for every *Banksia* species observed.

Id = depression index
Irm = index of recurved margin
Ie = epidermis index
Ih = hair index ($x10^2$ μm^2)
Sc = stomatal cuticle thickness (μm)
Uec = the thickness of the upper epidermis cuticle (μm)
Lec = the thickness of the lower epidermis cuticle (μm)
Rf = minimum of the range of annual rainfall of habitat (mm)

No	Species	Id	Irm	Ih	Ie	Sc	Uec	Lec	Rf
1	*B. praemorsa*	0.86 ±0.01	1.00	5.65 ±0.68	0.80 ±0.10	0.80 ±0.00	4.95 ±0.15	1.20 ±0.15	800
2	*B. ashbyi*	1.18 ±0.07	1.00	1.26 ±0.18	0.30 ±0.00	0.60 ±0.15	3.30 ±0.30	1.10 ±0.15	250
3	*B. integrifolia* var. *integrifolia*	0.00 ±0.00	1.00	2.67 ±0.26	1.00 ±0.00	0.80 ±0.00	3.45 ±0.30	1.10 ±0.15	800
4	*B. sceptrum*	1.05 ±0.04	1.00	0.40 ±0.05	0.40 ±0.10	0.80 ±0.00	1.80 ±0.15	0.90 ±0.15	300
5	*B. attenuata*	1.47 ±0.28	1.00	8.79 ±1.06	0.00	0.80 ±0.15	2.40 ±0.15	0.80 ±0.15	300
6	*B. conferta* var. *conferta*	0.00	1.00	4.31 ±0.21	1.20 ±0.10	0.90 ±0.15	3.00 ±0.45	0.90 ±0.15	1000
7	*B. caleyi*	2.76 ±0.29	1.00	5.12 ±0.12	0.00	0.80 ±0.00	4.50 ±0.30	0.90 ±0.15	550
8	*B. occidentalis*	0.00	1.08 ±0.06	15.03 ±1.84	1.70 ±0.10	0.80 ±0.00	2.40 ±0.30	0.80 ±0.00	350
9	*B. media*	0.91 ±0.02	1.00	5.50 ±0.46	0.70 ±0.00	0.80 ±0.15	2.85 ±0.15	0.90 ±0.15	300
10	*B. laevigata* ssp *laevigata*	0.00	1.21 ±0.03	7.92 ±0.59	2.00 ±0.10	0.80 ±0.00	2.85 ±0.15	0.90 ±0.15	400
11	*B. baxteri*	0.94 ±0.05	1.00	2.29 ±0.24	0.80 ±0.10	0.90 ±0.15	2.40 ±0.15	0.90 ±0.15	500
12	*B.serrata*	0.92 ±0.02	1.00	1.85 ±0.15	1.20 ±0.10	0.80 ±0.15	3.30 ±0.15	1.10 ±0.15	800

Table 2.5. continued

No	Species	Id	Im	Ih	Ie	Sc	Uec	Lec	Rf
13	*B. seminuda*	0.00	1.00	6.85 ±0.71	1.60 ±0.10	0.90 ±0.15	2.70 ±0.15	1.20 ±0.15	900
14	*B. lemanniana*	0.83 ±0.02	1.00	3.23 ±0.26	0.60 ±0.00	0.80 ±0.00	3.00 ±0.15	0.80 ±0.00	500
15	*B. baueri*	0.89 ±0.03	1.00	3.97 ±0.05	0.70 ±0.00	0.80 ±0.00	2.40 ±0.15	0.90 ±0.15	350
16	*B. robur*	1.66 ±0.03	1.00	1.08 ±0.05	0.90 ±0.00	0.80 ±0.00	3.30 ±0.30	0.90 ±0.15	800
17	*B. verticillata*	0.00	1.00	11.53 ±1.34	1.80 ±0.10	0.80 ±0.00	5.40 ±0.45	0.90 ±0.15	800
18	*B. spinulosa* var. *spinulosa*	0.00	1.00	13.80 ±1.25	3.30 ±0.10	0.50 ±0.00	2.70 ±0.15	0.90 ±0.15	600
19	*B. brownii*	0.00	1.00	13.99 ±1.16	1.90 ±0.10	0.50 ±0.00	2.10 ±0.15	0.80 ±0.00	600
20	*B. dryandroides*	0.00	1.38 ±0.06	7.43 ±0.76	1.30 ±0.10	0.50 ±0.00	3.90 ±0.30	0.60 ±0.00	700
21	*B. nutans* var. *cernuella*	0.00	2.46 ±0.28	17.22 ±0.64	0.00	0.60 ±0.00	2.10 ±0.15	0.90 ±0.15	350
22	*B. ericifolia* var. *ericifolia*	0.00	1.26 ±0.03	8.67 ±0.39	1.10 ±0.10	0.60 ±0.00	1.95 ±0.00	0.75 ±0.00	900
23	*B. aemula*	1.31 ±0.05	1.00	1.89 ±0.38	1.30 ±0.00	0.40 ±0.00	2.55 ±0.15	0.90 ±0.00	800

Figure 2.14. Graphs showing correlation between stomatal protection components of *Banksia* and minimum of the range of habitat annual rainfall.

A. Index depression vs. rainfall. The correlation coefficient -0.242 was found to be not significant ($p>0.1$).

B. Index recurved margin vs. rainfall. The correlation coefficient -0.177 was found to be not significant ($p>0.1$).

C. Index hair vs. rainfall. The correlation coefficient 0.022 was found to be not significant ($p>0.1$).

D. Index epidermis vs. rainfall. The correlation coefficient 0.143 was found to be not significant ($p>0.1$).

E. Stomatal cuticle vs. rainfall. The correlation coefficient 0.367 was found to be not significant ($p>0.05$).

F. Upper epidermis cuticle vs. rainfall. The correlation coefficient 0.016 was found to be not significant ($p>0.1$)

G. Lower epidermis cuticle vs. rainfall. The correlation coefficient 0.225 was found to be not significant ($p>0.1$).

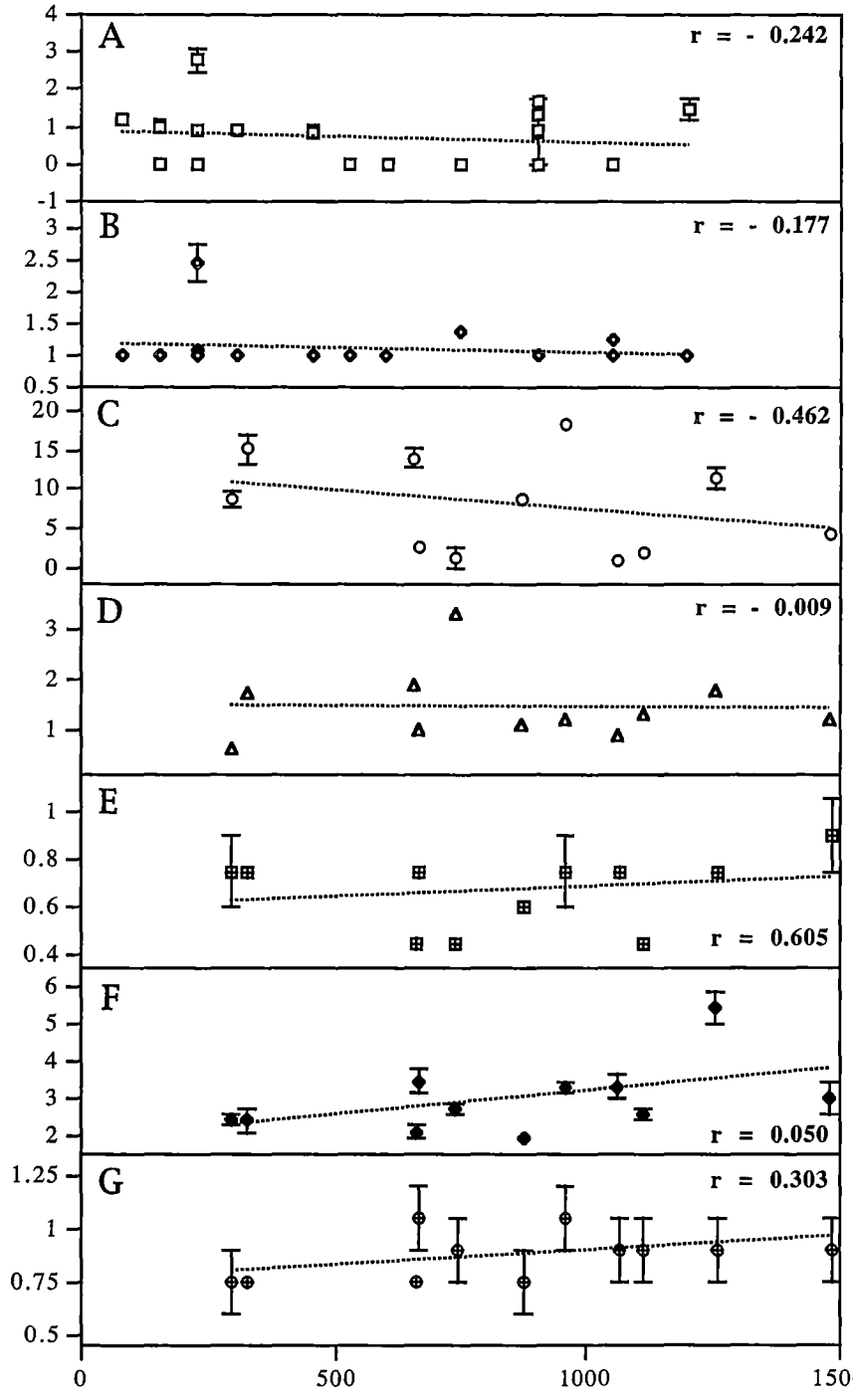

A
r = - 0.242
B
r = - 0.177
C
r = - 0.462
D
r = - 0.009
E
r = 0.605
F
r = 0.050
G
r = 0.303
0
500
1000
1500
Rainfall (mm)

Table 2.6. Index (IC) and cuticle component (CC) and the ranges of habitat climatic factors (generated using ESOCLIM) for every *Banksia* species observed.

Id = depression index; Irm = index of recurved margin; Ih = hair index (x10^2 µm^2); Ie = epidermis index; Sc = stomatal cuticle thickness (µm); Uec = thickness of upper epidermis cuticle (µm); Lec = thickness of lower epidermis cuticle (µm); Rf = the range of annual rainfall (mm); T = the range of annual temperature (°C); R = the range of daily radiation (Mj/m^2/day); Wr = the range of daily wind run (km/day)

Species	IC				CC			Climatic factors			
	Id	Irm	Ih	Ie	Sc	Uec	Lec	Rf	T	R	Wr
B. integrifolia var.*integrifolia*	0.00	1.00	2.67	1	0.75	3.45	1.05	666-1888	9-25.6	15.1-19.7	290.7-339.3
B. attenuata	1.47	1.00	8.79	0.60	0.75	2.40	0.75	291-1030	9.8-32.8	17.7-22.7	317.9-337.6
B. occidentalis	0.00	1.08	15.03	1.70	0.75	2.40	0.75	322-1002	9.8-23.1	16-18.8	342-368.3
B. serrata	0.92	1.00	1.85	1.20	0.75	3.30	1.05	957-1584	7.2-25.5	16.2-18.2	309.7-344.3
B. robur	1.66	1.00	1.08	0.90	0.75	3.30	0.90	1064-1616	13.9-27.1	18.3-20.6	281.4-334.3
B. verticillata	0.00	1.00	11.53	1.80	0.75	5.40	0.98	1256-1351	10.6-20.7	15.7	376.2-378.6
B. spinulosa var. *spinulosa*	0.00	1.00	13.80	3.30	0.45	2.70	0.90	741-2869	5.6-28.3	16.2-20.7	289.3-336.9
B. brownii	0.00	1.00	13.99	1.90	0.45	2.10	0.75	659-981	9-20.8	15.9-16.5	360.9-369.1
B. ericifolia var. *ericifolia*	0.00	1.26	8.67	1.10	0.60	1.95	0.75	873-1505	5.8-21.2	16.3-16.7	295.8-308.8
B. aemula	1.31	1.00	1.89	1.30	0.45	2.55	0.90	1115-1855	12.3-25.6	16.5-19.5	307.4-342.3

Figure 2.15. Graphs showing correlation between stomatal protection components of *Banksia* and estimated minimum of the range of habitat annual rainfall.

A. Index depression vs. rainfall. The correlation coefficient 0.134 was found to be not significant ($p>0.1$).

B. Index recurved margin vs. rainfall. The correlation coefficient -0.051 was found to be not significant ($p>0.1$).

C. Index hair vs. rainfall. The correlation coefficient -0.462 was found to be not significant ($p>0.1$).

D. Index epidermis vs. rainfall. The correlation coefficient -0.009 was found to be not significant ($p>0.1$).

E. Stomatal cuticle vs. rainfall. The correlation coefficient 0.464 was found to be not significant ($p>0.05$).

F. Upper epidermis cuticle vs. rainfall. The correlation coefficient 0.605 was found to be not significant ($p>0.05$).

G. Lower epidermis cuticle vs. rainfall. The correlation coefficient 0.303 was found to be not significant ($p>0.1$).

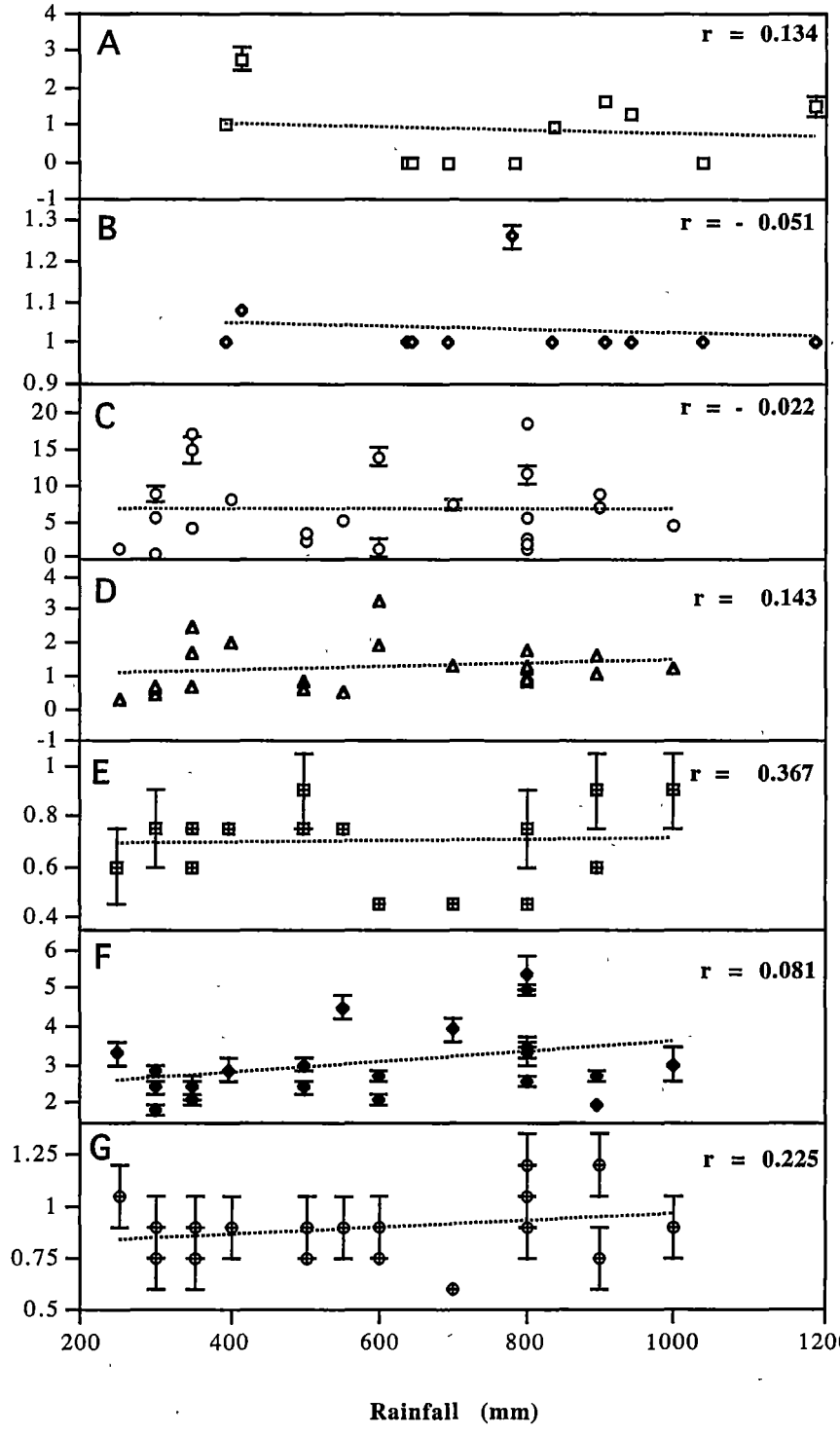

A
r = 0.134
B
r = - 0.051
C
r = - 0.022
D
r = 0.143
E
r = 0.367
F
r = 0.081
G
r = 0.225
200
400
600
800
1000
1200
Rainfall (mm)

Figure 2.16. Graphs showing correlation between stomatal protection components of *Banksia* and estimated maximum of the range of habitat daily temperatures.

A. Index depression vs. temperature. The correlation coefficient 0.643 was found to be significant ($p<0.05$).

B. Index recurved margin vs. temperature. The correlation coefficient 0.402 was found to be not significant ($p>0.1$).

C. Index hair vs. temperature. The correlation coefficient -0.264 was found to be not significant ($p>0.1$).

D. Index epidermis vs. temperature. The correlation coefficient -0.142 was found to be not significant ($p>0.1$).

E. Stomatal cuticle vs. temperature. The correlation coefficient 0.208 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle vs. temperature. The correlation coefficient -0.142 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle vs. temperature. The correlation coefficient 0.262 was found to be not significant ($p>0.1$).

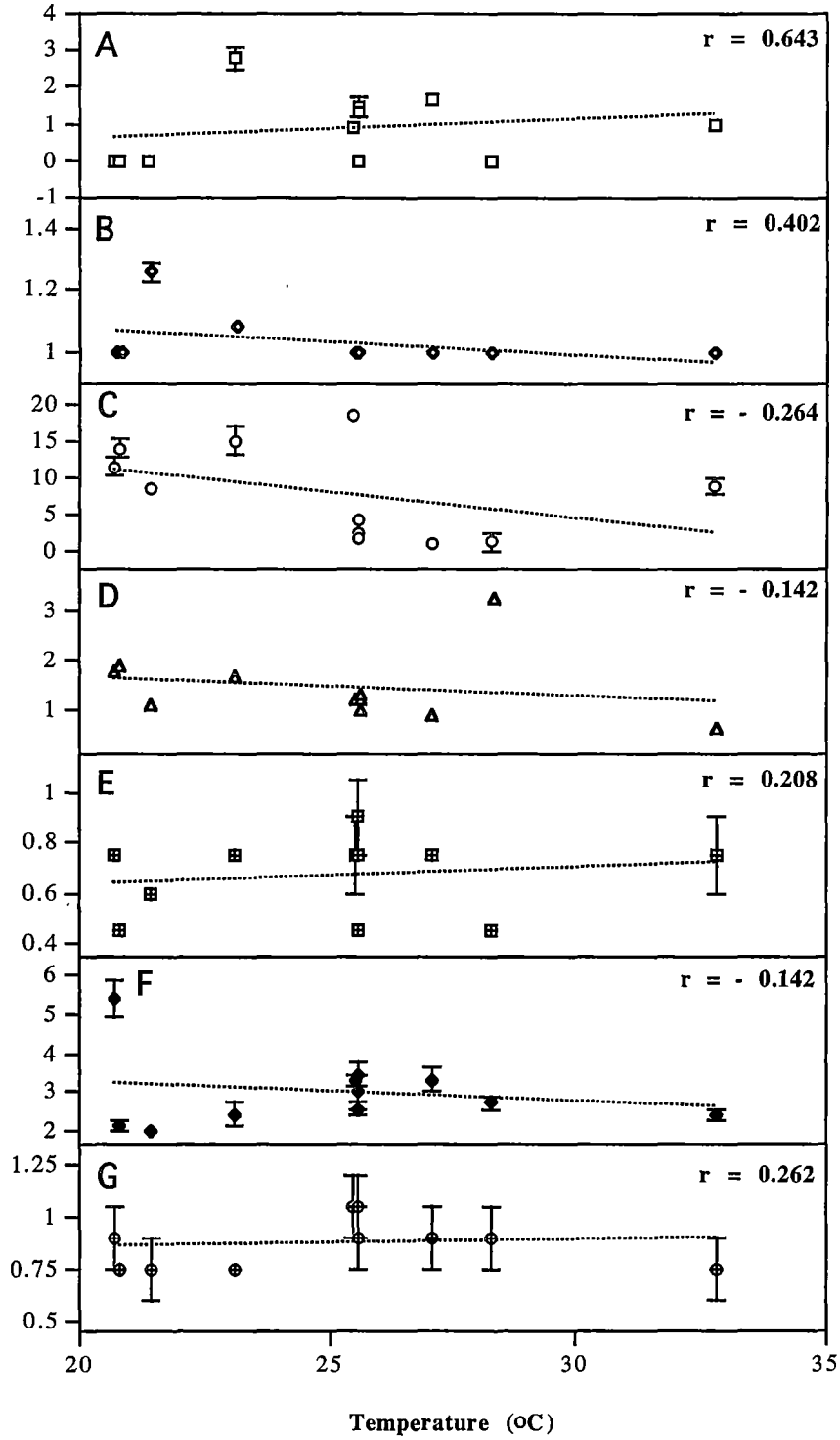

A
r = 0.643
B
r = 0.402
C
r = - 0.264
D
r = - 0.142
E
r = 0.208
F
r = - 0.142
G
r = 0.262
Temperature (oC)

Figure2.17. Graphs show correlation between stomatal protection components of *Banksia* and estimated maximum of the range of habitat daily radiation.

A. Index depression vs. radiation. The correlation coefficient 0.604 was found to be significant ($p>0.05$).

B. Index recurved margin vs. radiation. The correlation coefficient -0.358 was found to be not significant ($p>0.1$).

C. Index hair vs. radiation. The correlation coefficient -0.273 was found to be not significant ($p>0.1$).

D. Index epidermis vs. radiation. The correlation coefficient -0.121 was found to be not significant ($p>0.1$).

E. Stomatal cuticle vs. radiation. The correlation coefficient 0.310 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle vs. radiation. The correlation coefficient -0.155 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle vs. radiation. The correlation coefficient 0.277 was found to be significant ($p<0.1$).

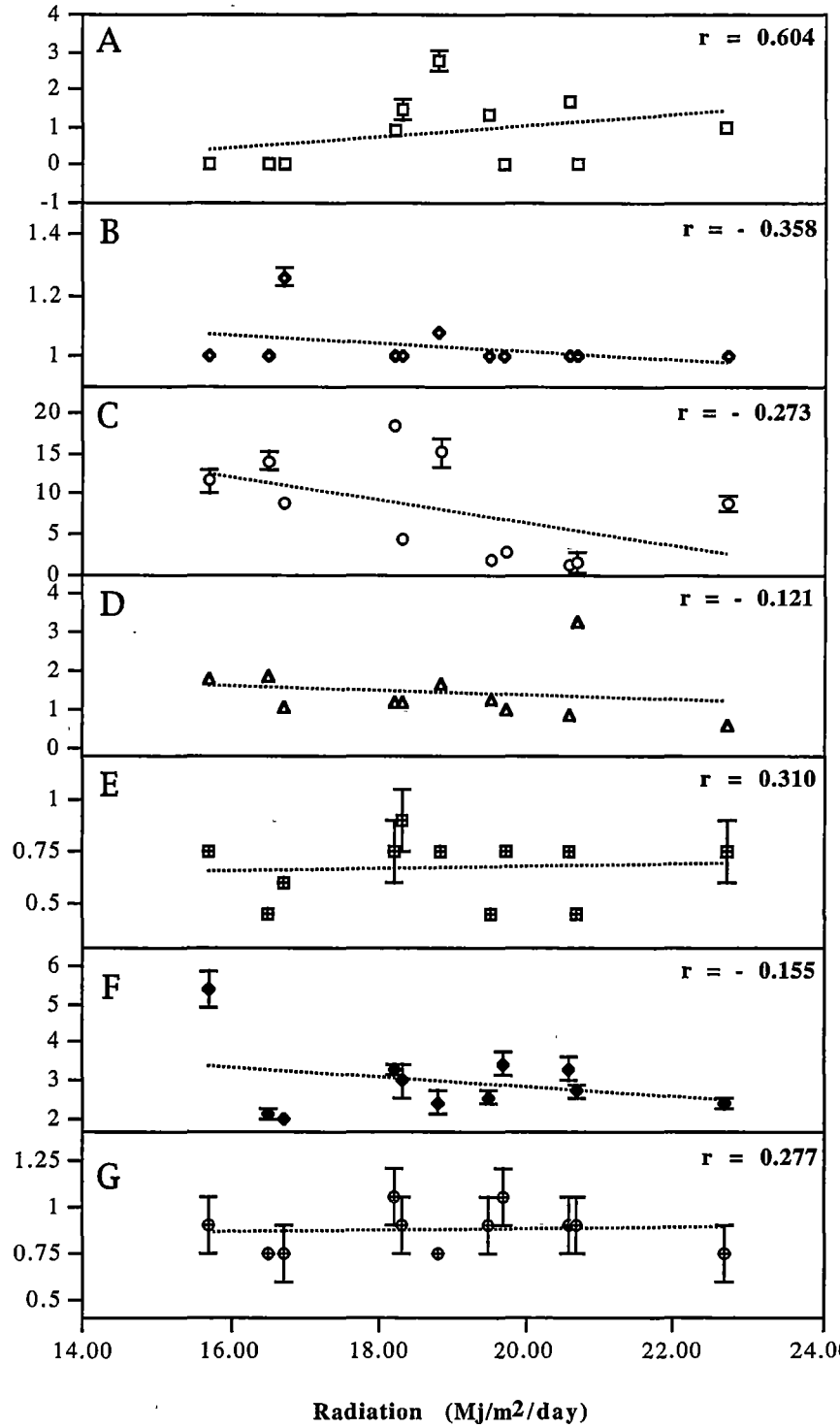

A
r = 0.604
B
r = - 0.358
C
r = - 0.273
D
r = - 0.121
E
r = 0.310
F
r = - 0.155
G
r = 0.277
14.00
16.00
18.00
20.00
22.00
24.00
Radiation (Mj/m2/day)

Figure 2.18. Graphs showing correlation between stomatal protection components of *Banksia* and estimated maximum of the range of daily wind run of the habitat.

A. Index depression vs. wind run. The correlation coefficient -0.286 was found to be not significant ($p>0.1$).

B. Index recurved margin vs. wind run. The correlation coefficient 0.528 was found to be not significant ($p>0.1$).

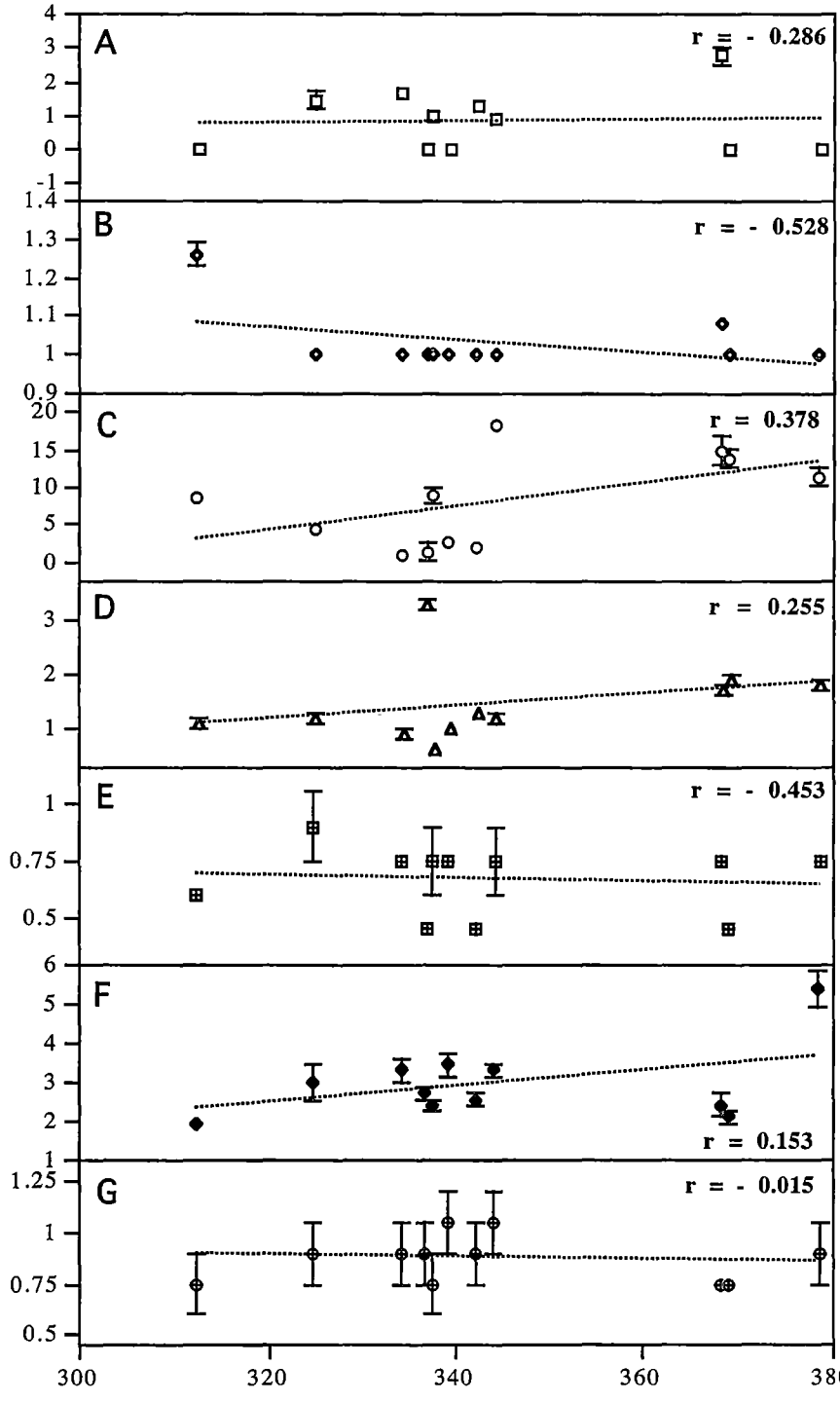
C. Index hair vs. wind run. The correlation coefficient 0.378 was found to be not significant ($p>0.1$).

D. Index epidermis vs. wind run. The correlation coefficient 0.255 was found to be not significant ($p>0.1$).

E. Stomatal cuticle vs. wind run. The correlation coefficient -0.453 found to be not significant ($p>0.1$).

F. Upper epidermis cuticle vs. wind run. The correlation coefficient 0.153 found to be not significant ($p>0.1$).

G. Lower epidermis cuticle vs. wind run. The correlation coefficient -0.015 found to be not significant ($p>0.1$).


A
r = - 0.286
B
r = - 0.528
C
r = 0.378
D
r = 0.255
E
r = - 0.453
F
r = 0.153
G
r = - 0.015
300
320
340
360
380
Wind run (km/day)

In order to investigate which dimensions of the characters give large contributions to the character performances a correlation analysis between every dimension with the habitat climatic factor was applied (Table 2.7).

Table2.7. Correlation (coefficient value and probability) between character dimensions of *Banksia* species and habitat climatic factors Dim = dimensions; Rft = minimum of the range of annual rainfall for all species observed; Rf = minimum of the range of annual rainfall for estimated rainfall; T = maximum of the range of annual temperature; R = maximum of the range of daily radiation; Wr= maximum of the range of daily wind run; c = count; r = correlation coefficient; p = probability; Id = depression index; ls and ws = length and width of depression surface area; lm and wm = length and width of depression median area; dd = depression depth; Irm = index of recurved margin; Ih = hair index; hld and hdd = hair length and diameter of depression hairs; hle and hde = hair length and diameter of epidermis hairs; Ie = epidermis index; de = epidermis hair density; dd = depression hair density; ds = stomate density; Sc, Uec and Lec = thickness of stomatal cuticle and upper and lower epidermis cuticle.

Dim	Rft			Rf			T		R		Wr	
	c	r	p	c	r	p	r	p	r	p	r	p
Id	23	-0.372	>0.1	11	0.106	>0.1	0.646	<0.05	0.579	>0.05	-0.282	>0.1
ls	23	-0.333	>0.1	11	0.163	>0.1	0.607	>0.05	0.532	>0.1	-0.267	>0.1
ws	23	-0.337	>0.1	11	0.149	>0.1	0.618	>0.05	0.539	>0.1	-0.267	>0.1
wsxls	23	-0.339	>0.1	11	0.105	>0.1	0.646	<0.05	0.558	>0.05	-0.263	>0.1
lm	23	-0.329	>0.1	11	0.157	>0.1	0.614	>0.05	0.541	>0.1	-0.270	>0.1
wm	23	-0.330	>0.1	11	0.142	>0.1	0.627	>0.05	0.554	>0.05	-0.273	>0.1
wmxlm	23	-0.326	>0.1	11	0.107	>0.1	0.654	<0.05	0.582	>0.05	-0.274	>0.1
dd	23	-0.282	>0.1	11	0.092	>0.1	0.648	<0.05	0.602	>0.05	-0.325	>0.1
Irm*	23	-0.153	>0.1	11	-0.477	>0.1	-0.295	>0.1	-0.135	>0.1	0.165	>0.1
Ih	23	-0.025	>0.1	11	-0.461	>0.1	-0.268	>0.1	-0.276	>0.1	0.378	>0.1
hld	23	-0.051	>0.1	11	-0.387	>0.1	-0.117	>0.1	-0.182	>0.1	0.067	>0.1
hdd	23	-0.288	>0.1	11	-0.352	>0.1	-0.232	>0.1	-0.190	>0.1	0.524	>0.1
hle	23	-0.097	>0.1	11	0.348	>0.1	0.164	>0.1	0.103	>0.1	-0.163	>0.1
hde	23	-0.146	>0.1	11	0.351	>0.1	0.167	>0.1	0.110	>0.1	-0.166	>0.1
Ie	23	0.143	>0.1	11	-0.090	>0.1	-0.142	>0.1	-0.122	>0.1	0.255	>0.1
fe	23	-0.292	>0.1	11	0.130	>0.1	0.451	>0.1	0.360	>0.1	-0.224	>0.1
fd	23	0.187	>0.1	11	-0.096	>0.1	-0.133	>0.1	-0.058	>0.1	0.261	>0.1
fs	23	-0.296	>0.1	11	-0.377	>0.1	0.541	>0.1	0.628	>0.05	-0.296	>0.1
Sc	23	-0.369	>0.05	11	-0.606	>0.05	-0.208	>0.1	-0.310	>0.1	-0.453	>0.1
Uec	23	-0.021	>0.1	11	-0.055	>0.1	-0.144	>0.1	0.154	>0.1	-0.149	>0.1
Lec	23	-0.225	>0.1	11	-0.31	>0.1	-0.270	>0.1	-0 286	>0.1	-0.001	>0.1

* has relative values

The correlation analysis for total rainfall (Rft) and estimated rainfall (Rf) revealed different results (Table 2.7). Seemingly, the former gives better results due to the higher number of species represented. There was a negative trend for the correlation of every character observed, except Ie. The index values decreased with increasing minimum of the range of annual rainfall. Statistically, however, the coefficient correlations were not significant, except that of Sc ($p<0.05$). Presumably, this structure has a large role in inhibiting excessive transpiration from the stomates themselves.

In response to the maximum of the range of annual temperatures, stomatal depression showed a significant correlation, *i.e.* -0.646 ($p<0.05$)(Table 2.7). Presumably the hidden stomates are less exposed to high temperatures, hence they do not transpire excessively. The more hidden the stomate positions, the more protected they are. This idea is supported by the finding that the areas of surface and median plates and depression depth also had significant correlations ($p<0.05$).

Hairs are prominent structures in *Banksia* leaves, however, their presence does not show a significant association with any habitat climatic factor (Table 2.7). Regardless of the significance, this finding showed that there was a negative trend of correlation between hair index and the minimum of the range of annual rainfall. Meanwhile, a positive trend of correlation between the hair index and maximum of the range of daily wind run was observed. This probably means that hairs function as a stomatal cover, which inhibits the breeze sweeping water vapour away.

The cuticle thickness of the stomates, and the upper and lower epidermis did not reveal any correlation ($p>0.1$) with any of the habitat climatic factors observed (Table 2.7). Among them, seemingly the stomatal cuticle thickness showed the greatest tendency for having significant correlation ($r=-0.606$; $p=0.063$). Presumably, in this genus stomatal cuticles play some role as a barrier from the effect of the dryness caused by low rainfall.

C. Character ranges

Analysis of variance for the characters measured (Table 2.8) was applied. The results show that there are highly significant differences in the value of every index calculated among the species observed. Characters assumed to increase the boundary layers were found to be very diverse both qualitatively and quantitatively (see Table 2.8 and Figures 2.13 a-h).

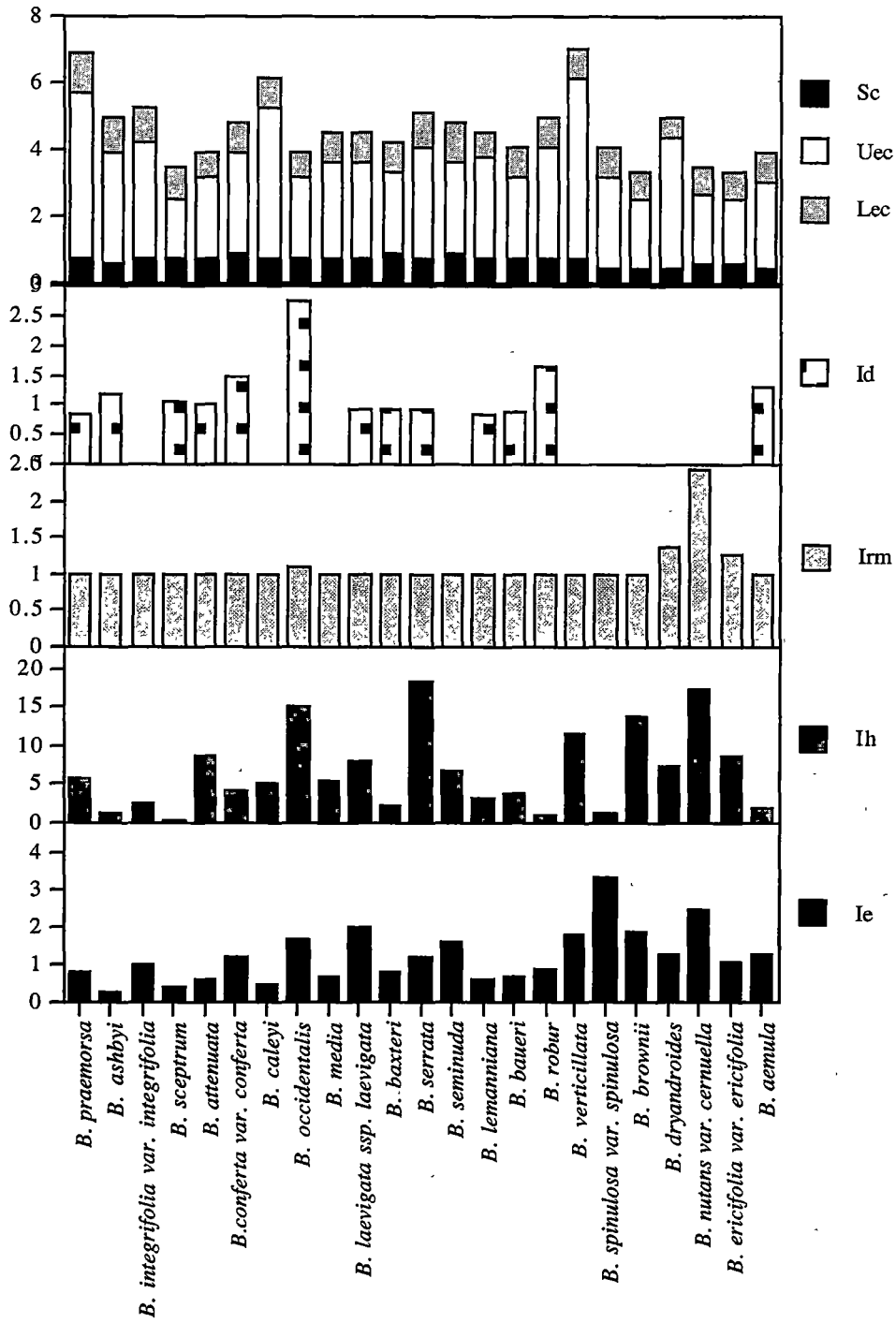


Figure 2.19. Graphs describing the variation of stomatal protection components in *Banksia* species.
Id = depression index; Irm = index of recurved margin (value 1 equal to 0, see pages 36-37); Ih= hair index ($10^2\mu m^2$); Ie = epidermis index; Sc = stomatal cuticle thickness (μm); Uec = upper epidermis cuticle (μm); lec = lower epidermis cuticle (μm).

Table 2.8. Significant levels of every index value and cuticle thickness among *Banksia* species observed.

Indices and cuticle thickness	F-test	Probability	Transformation
Depression index (Id)	26.461	0.0001	$Log_{10}(Y+1)$
Index of recurved margin (Irm)	28.570	0.0001	1/Y
Hair index (Ih)	88.29	0.0001	$log_{10}Y$
Epidermis index (Ie)	103.54	0.0001	untransformed
Stomatal cuticle (Sc)	13.34	0.0001	untransformed
Upper epidermis cuticle (Uec)	6.51	0.0001	untransformed
Lower epidermis cuticle (Lec)	2.18	0.0054	untransformed

Tables 2.9 a-h. Comparisons of mean values among *Banksia* species observed in every index calculated. These tables describe more about the variety of the values of every index calculated among species observed.

a. Depression index (Id)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
B. caleyi	5	2.721	A
B. robur	5	1.661	B
B. attenuata	5	1.413	B C
B. aemula	5	1.305	B C
B. ashbyi	5	1.176	C D
B. sceptrum	5	1.044	C D E
B. baxteri	5	0.941	D E
B. serrata	5	0.924	D E
B. media	5	0.907	D E
B. baueri	5	0.893	E
B. praemorsa	5	0.862	E
B. lemanniana	5	0.825	E
B. conferta var. *conferta**	5	1.000	
B.laevigata ssp. *laevigata* *	5	1.000	
*B. brownii**	5	1.000	
*B. occidentalis**	5	1.000	
B. nutans var. *cernuella**	5	1.000	
B. ericifolia var. *ericifolia**	5	1.000	
B. integrifolia var. *integrifolia**	5	1.000	
*B. seminuda**	5	1.000	
*B. dryandroides**	5	1.000	
B. spinulosa var. *spinulosa**	5	1.000	
B. verticillata *	5	1.000	

* not included in the analysis

b. Index of recurved margin (Irm)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
B. ericifolia var. *ericifolia*	5	2.344	A
B. nutans var. *cernuella*	5	1.371	B
B. dryandroides	5	1.257	B C
B. laevigata ssp. *laevigata*	5	1.204	B C
B. occidentalis	5	1.063	C
*B. baxteri**	5	0.000	
*B. attenuata**	5	0.000	
*B. brownii**	5	0.000	
*B. aemula**	5	0.000	
*B. ashbyi**	5	0.000	
B. integrifolia var. *integrifolia**	5	0.000	
*B. baueri**	5	0.000	
*B. lemanniana**	5	0.000	
*B. media**	5	0.000	
*B. caleyi**	5	0.000	
B. conferta var. *conferta**	5	0.000	
*B. praemorsa**	5	0.000	
*B. robur**	5	0.000	
*B. sceptrum**	5	0.000	
*B. seminuda**	5	0.000	
*B. serrata**	5	0.000	
B. spinulosa var. *spinulosa**	5	0.000	
*B. verticillata**	5	0.000	

* not included in the analysis

c. Hair index (Ih)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
B. nutans var. *cernuella*	5	17.178	A
B. occidentalis	5	14.537	A B
B. brownii	5	13.764	A B
B. spinulosa var. *spinulosa*	5	13.588	A B
B. verticillata	5	11.199	A B C
B. attenuata	5	8.631	B C D
B. ericifolia var. *ericifolia*	5	8.570	B C D
B. laevigata ssp. *laevigata*	5	7.830	C D
B. dryandroides	5	7.234	C D E
B. seminuda	5	6.702	C D E F
B. praemorsa	5	5.477	D E F
B. media	5	5.426	D E F G
B caleyi	5	5.116	D E F G
B. conferta var. *conferta*	5	4.291	E F G H
B. baueri	5	3.951	F G H
B. lemanniana	5	3.180	G H I
B. integrifolia var. *integrifolia*	5	2.620	H I J
B. baxteri	5	2.238	I J
B. serrata	5	1.822	J K
B. aemula	5	1.760	J K
B. ashbyi	5	1.215	K
B. robur	5	1.075	K
B. sceptrum	5	0.385	L

d. Epidermis index (Ie)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
B. spinulosa var. *spinulosa*	5	3.340	A
B. nutans var. *cernuella*	5	2.480	B
B. laevigata ssp. *laevigata*	5	1.980	C
B. brownii	5	1.940	C D
B. occidentalis	5	1.720	C D
B. seminuda	5	1.620	C D E
B. verticillata	5	1.600	D E
B. aemula	5	1.340	E F
B. dryandroides	5	1.280	E F
B. serrata	5	1.160	F G
B. conferta var. *conferta*	5	1.600	F G
B. ericifolia var. *ericifolia*	5	1.080	F G
B. integrifolia var. *integrifolia*	5	1.000	F G H
B. robur	5	0.860	G H I
B. baxteri	5	0.840	G H I
B. praemorsa	5	0.840	G H I
B. baueri	5	0.680	H I J
B. media	5	0.660	H I J
B. attenuata	5	0.620	I J
B. lemaniana	5	0.540	I J
B. caleyi	5	0.440	J
B. sceptrum	5	0.400	J
B. ashbyi	5	0.340	J

e. Stomatal cuticle (Sc)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
B. verticillata	5	5.410	A
B. praemorsa	5	5.004	A B
B. caleyi	5	4.460	A B C
B. dryandroides	5	3.856	B C D
B. integrifolia var. *integrifolia*	5	3.470	C D E
B. serrata	5	3.370	C D E F
B. robur	5	3.328	C D E F G
B. ashbyi	5	3.272	C D E F G
B. conferta var. *conferta*	5	2.938	D E F G H
B. lemanniana	5	2.930	D E F G H
B. media	5	2.852	D E F G H
B. laevigata ssp. *laevigata*	5	2.802	D E F G H
B. spinulosa var. *spinulosa*	5	2.714	D E F G H
B. seminuda	5	2.686	D E F G H
B. aemula	5	2.616	D E F G H
B. baueri	5	2.474	E F G H
B. occidentalis	5	2.382	E F G H
B. baxteri	5	2.360	E F G H
B. attenuata	5	2.340	E F G H
B. brownii	5	2.172	E F G H
B. nutans var. *cernuella*	5	2.040	F G H
B. ericifolia var. *ericifolia*	5	2.020	G H
B. sceptrum	5	1.802	H

f. Upper epidermis cuticle (Uec)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
B. baxteri	5	0.824	A
B. seminuda	5	0.824	A
B. conferta var. *conferta*	5	0.824	A
B. sceptrum	5	0.750	A B
B. praemorsa	5	0.750	A B
B. robur	5	0.750	A B
B. caleyi	5	0.750	A B
B. laevigata ssp. *laevigata*	5	0.750	A B
B. lemanniana	5	0.750	A B
B. occidentalis	5	0.750	A B
B. integrifolia var. *integrifolia*	5	0.750	A B
B. baueri	5	0.750	A B
B. verticillata	5	0.750	A B
B. media	5	0.676	A B C
B. serrata	5	0.676	A B C
B. attenuata	5	0.676	A B C
B. ashbyi	5	0.602	A B C D
B. nutans var. *cernuella*	5	0.602	A B C D
B. ericifolia var. *ericifolia*	5	0.528	B C D
B. spinulosa var. *spinulosa*	5	0.454	C D
B. aemula	5	0.454	C D
B. brownii	5	0.380	D
B. dryandroides	5	0.380	D

g. Lower epidermis cuticle (Lec)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
B. seminuda	5	1.274	A
B. praemorsa	5	1.122	A B
B. serrata	5	0.974	A B C
B. ashbyi	5	0.972	A B C
B.integrifolia var.*integrifolia*	5	0.972	A B C
B. media	5	0.898	A B C
B. sceptrum	5	0.898	A B C
B. conferta var. *conferta*	5	0.898	A B C
B. aemula	5	0.898	A B C
B. robur	5	0.898	A B C
B. laevigata ssp. *laevigata*	5	0.898	A B C
B. baueri	5	0.898	A B C
B. spinulosa var. *spinulosa*	5	0.826	A B C
B. caleyi	5	0.824	A B C
B. baxteri	5	0.824	A B C
B. verticillata	5	0.824	A B C
B. occidentalis	5	0.824	A B C
B. lemanniana	5	0.750	A B C
B. attenuata	5	0.750	A B C
B. ericifolia var. *ericifolia*	5	0.676	B C
B. nutans var. *cernuella*	5	0.676	B C
B. brownii	5	0.676	B C
B. dryandroides	5	0.528	C

It is obvious that the values of every character measured and calculated varied among species observed. Some characters seemed to be more heterogeneous than

others (Table 2.9a-g). Figure 2.19 clearly displays the component number and the values of every component in every species observed. Furthermore, the graphs also show the variation of stomatal protection components for every species observed.

Stomatal depression is assumed to be an important character in *Banksia.* However, 47.8% of the species observed do not have it (Table 2.9a). Meanwhile 43% of the species with stomatal depressions (*e.g. B. serrata, B. baueri* and *B. media*) had a low level depression index. These species tended to occupy areas which had minimum of the range of annual rainfall ranging from 300-800 mm. *B. caleyi* and *B. robur,* which had high and medium values of depression index, inhabited areas with minimum of the range of annual rainfall 550 and 800 mm, respectively. Statistical analysis showed that depression index does not directly associate with the minimum of the range of annual rainfall of the habitat ($p>0.1$). However, it is significantly correlated with maximum of the range of annual temperatures ($p<0.05$).

It is assumed that revolute margins are possibly important in protecting stomates from dryness. In *Banksia,* however, it was found that they were not prominent. Among species observed, only 22% possessed this character with little variation (Table 2.9b). Statistical analysis also suggested that there was no significant correlation between the values of the index of revolute margin and minimum of the range of annual rainfall ($p>0.1$)(Table 2.7).

Hairs are prominent characters in *Banksia* especially on the abaxial surface. All of the species observed possessed this structure, but the hairs varied in size and shape (Table 2.9c). More than 17% of the species observed had high values of hair index (*e.g. B. occidentalis, B. brownii* inhabit areas with minimum of the range of annual rainfall of 300 - 600 mm). Species with medium (*e.g. B. dryandroides, B. attenuata*) and low (*e.g. B. caleyi, B. media*) hair index values tend to inhabit areas with minimum of the range of rainfall of 300 - 900 mm and 250 - 1000 of mm, respectively. However, it appears that the hair index values could not be easily associated with dryness of the species habitat ($r = 0.025$)(Table 2.7).

Epidermis index values varied among species observed (Table 2.9d). Species with high epidermis index values, namely *B. spinulosa* var. *spinulosa* and *B. nutans* var. *cernuella* tended to occupy habitats with a minimum of the range of annual rainfall of 350 - 600 mm, while those with medium (*e.g. B. brownii, B. seminuda*) and low (*e.g. B. serrata, B. baxteri*) epidermis index values inhabit places with a minimum of the range of annual rainfall of 350 - 900 mm and 250 - 1000 mm, respectively. This may

suggest that species with low epidermis index values are more tolerant to habitat dryness than those with higher values.

The thickness of the stomatal cuticle among *Banksia* species was very diverse compared to the two other kinds of cuticles (Table 2.9e). Among them, *B. verticillata, B. praemorsa,* and *B. caleyi* possessed the thickest stomatal cuticle. *B. caleyi, B. dryandroides, B. integrifolia* var. *integrifolia, B. serrata, B. robur* and *B. ashbyi* had medium thickness and the rest of the species (65%) *e.g. B. media, B. lemanniana, B. brownii* had the thinnest cuticles. The variety in the stomatal cuticle thickness tended to be associated with the minimum of the range of annual rainfall of the habitat (r=-0.606; p=0.063) (Table 2.7). This structure may play a role in stomatal protection from the dryness caused by low rainfalls.

The upper epidermis cuticle thickness seems to be more homogeneous than the stomatal cuticle thickness (Table 2.9f). Most of the species observed (56.5%) e.g. *B. baxteri, B. sceptrum, B. lemanniana,* had thick cuticles. They inhabited areas with a minimum of the range of annual rainfall of 300 - 800 mm. Species with a medium thickness (*B. serrata, B. ashbyi*) tended to inhabit areas with a minimum of the range of annual rainfall ranging from 250-800 mm. Meanwhile, species with the thinnest cuticles (*B. aemula, B. brownii*) inhabited places with minimum of the range of annual rainfall of 350-800 mm. It is obvious that the variation of the thickness of the upper epidermis cuticles is not associated with the habitat annual rainfall of the habitat. None of the other habitat climatic factors observed indicated any tendency to correlate with the cuticles (Table 2.7).

The lower epidermis cuticle thickness among species observed was more homogeneous than that of the upper epidermis cuticle (Table 2.9g). More than 70% of species observed (*e.g. B. serrata, B. sceptrum, B. robur*) had a medium thickness of the lower epidermis cuticle. They inhabited areas with a minimum of the range of annual rainfall ranging from 350-800 mm. Species with a thick lower epidermis cuticle tend to inhabit areas with 800-900 mm minimum of the range of annual rainfall. The rest of the species with the thinnest cuticles are adapted to live in areas with a minimum range of annual rainfall of 350-800 mm. It is clearly shown by these figures that the variation of the thickness of the lower epidermis cuticle does not reflect the correlation of climatic habitat factors, especially rainfall (Table 2.7).

2.3.2. *Grevillea*

A. Leaf Morphology

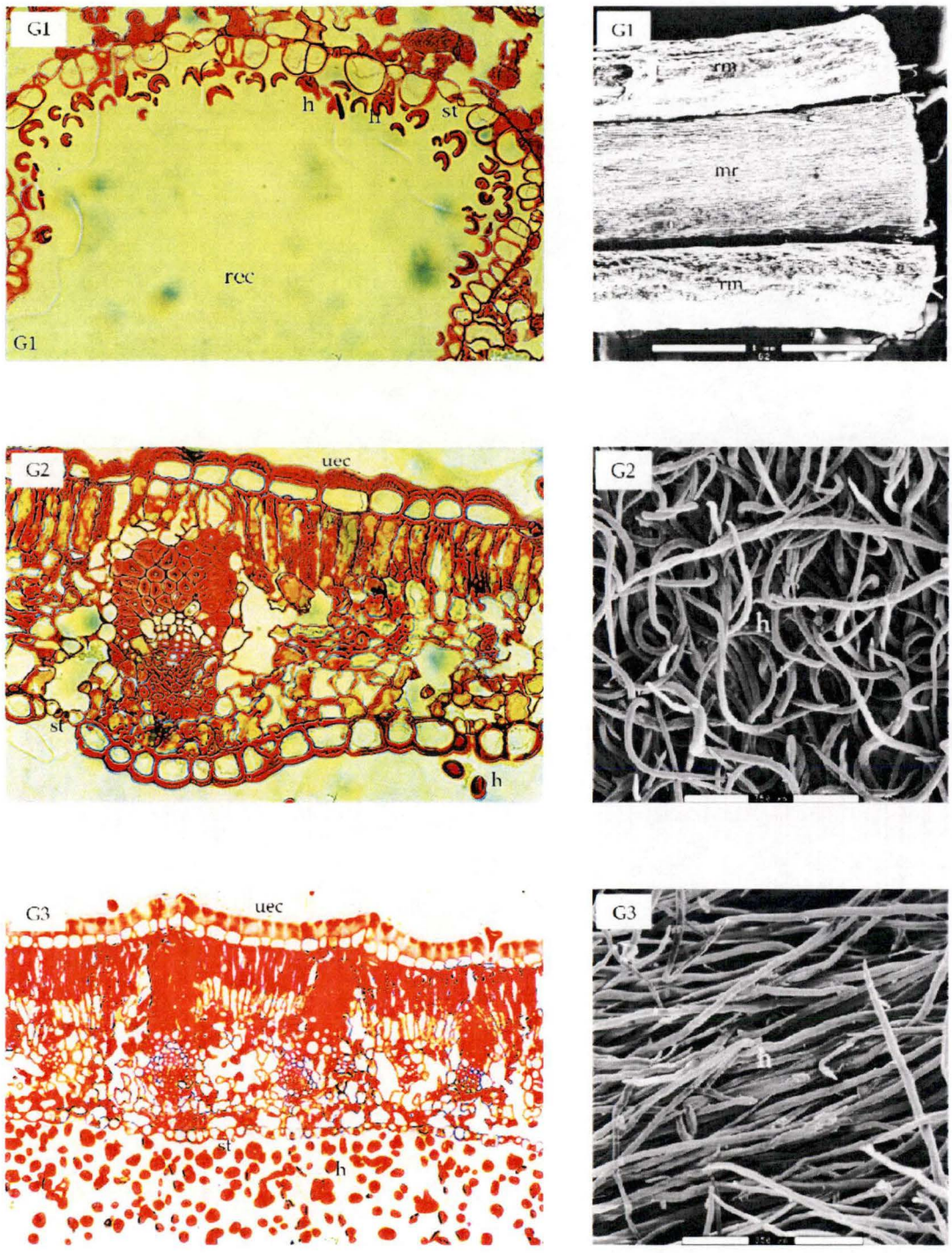
Leaf cross sections of some *Grevillea* species (Figure 2.20a-f) showed some similarities in their characters, namely the presence of T-shaped hairs on the lower surface, single stomatal depressions and obvious recurved margins. In some species (*e.g. G. lavandulacea* and *G. spinosissima*) T-shaped hairs seem to provide more canopies to the stomates and the neighbouring epidermis. Unlike *Banksia* leaves, the *Grevillea* leaves did not possess any prominent epidermal depression where stomates were collectively protected. *Grevillea* leaves, however, showed stomatal depressions surrounded by a very developed epidermis covered by a thick cuticle (*e.g.* Figure 2.20c-G7,G8). In contrast, the cuticle of the cells surrounding stomatal pores was not as thick as that on the epidermis.

Most of the leaves observed had recurved margins which varied from shortly recurved to revolute. Some arid habitat species (*e.g. G. lavandulacea* and *G. nudiflora*) showed very revolute leaf margins, which almost enclosed the whole undersurface areas (Figure 2.20b-G6). The roles of all the characters described above in relation to the habitat factors was investigated.

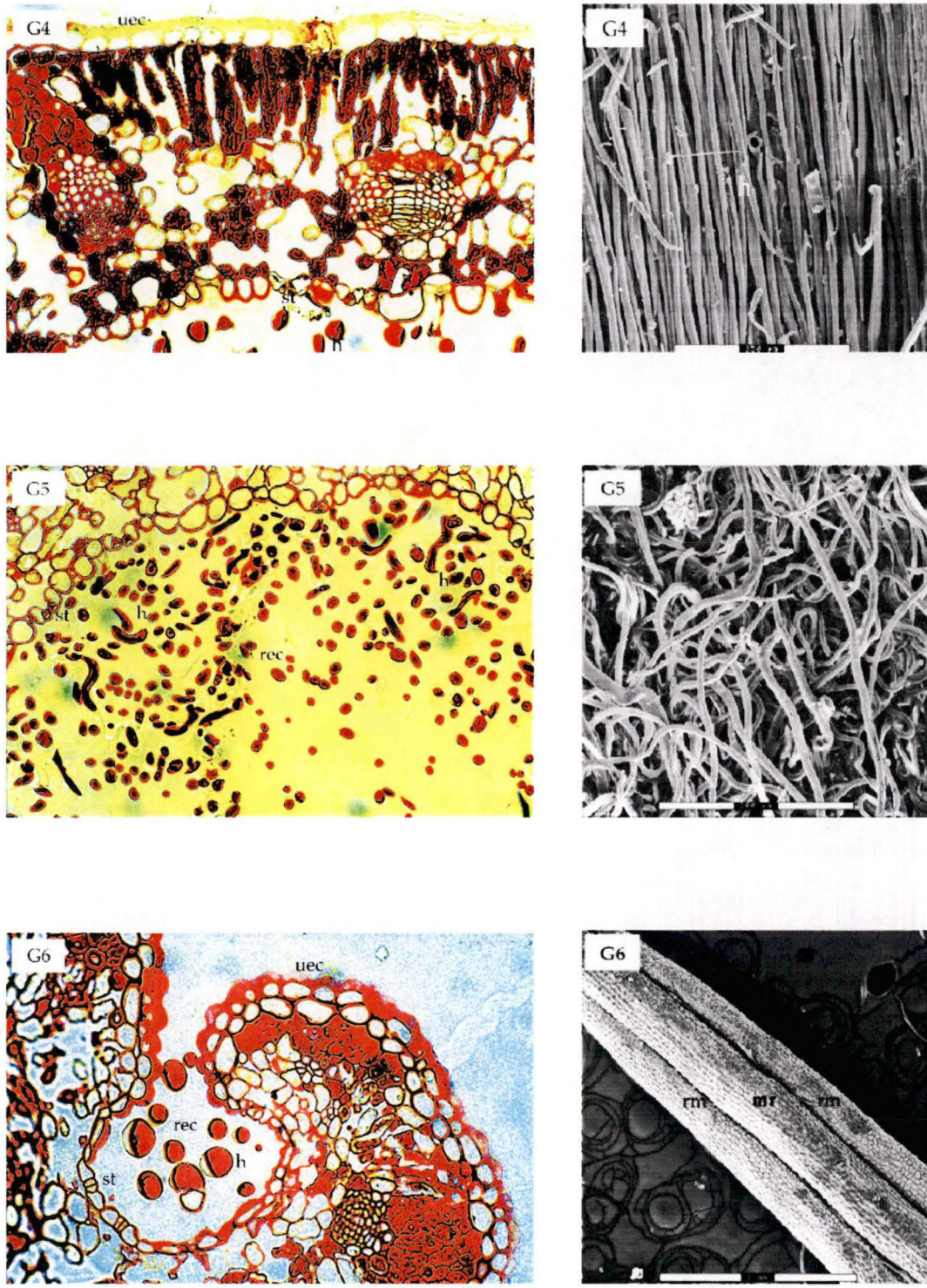
Morphological studies were conducted by observing the cross sections of leaves and ESEM images of the lower surface of leaves of *Grevillea* species. The following figures describe in detail the characters observed in every species.

B. Correlations

Correlations between components of stomatal protection and the minimum of the range of annual rainfall were investigated to determine whether there was a correlation between them. The lack of other climatic data for every species observed, however, was a large barrier to understanding more about plant and habitat correlations. An attempt to solve the problem was made by designing a correlation model using estimated climatic factors, which were generated from herbarium locality data using ESOCLIM analysis, with the character values.

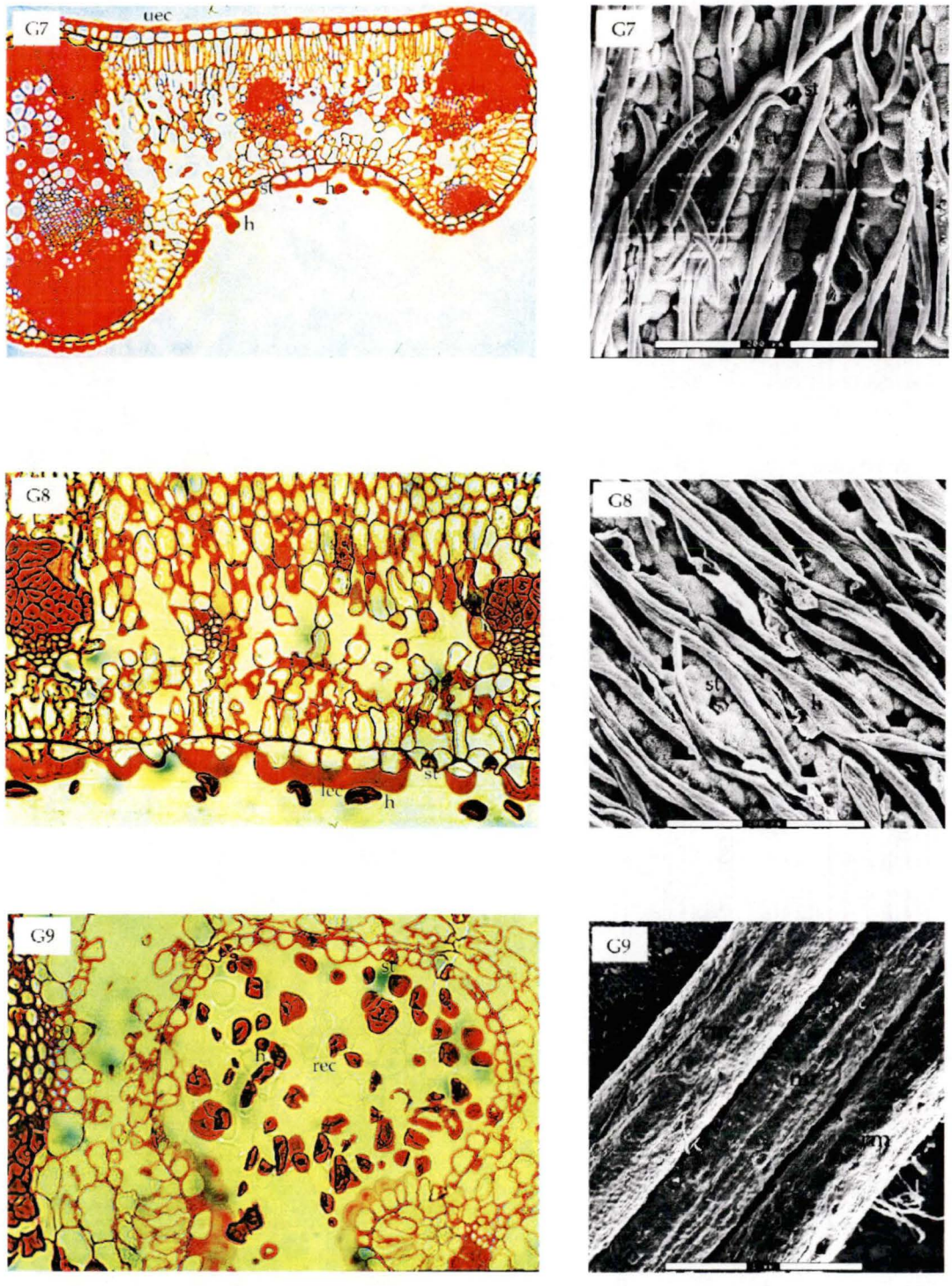


Figures2.20a. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *Grevillea johnsonii* (G1-left, 320x), *G. aquifolium* (G2-left, 320x), and *G. aspera* (G3-left, 160x). Note the presence of a thick upper epidermis cuticle in *G. aquifolium* and *G. aspera* and stomates protected by dense hairs. Hairs in *G. johnsonii* are covered by leaf margin recurvation. Stomates in *G. johnsonii* are protected by hairs and leaf recurvations.
h = hairs; rec = leaf recurvation; st = stomate; uec = upper epidermis cuticles

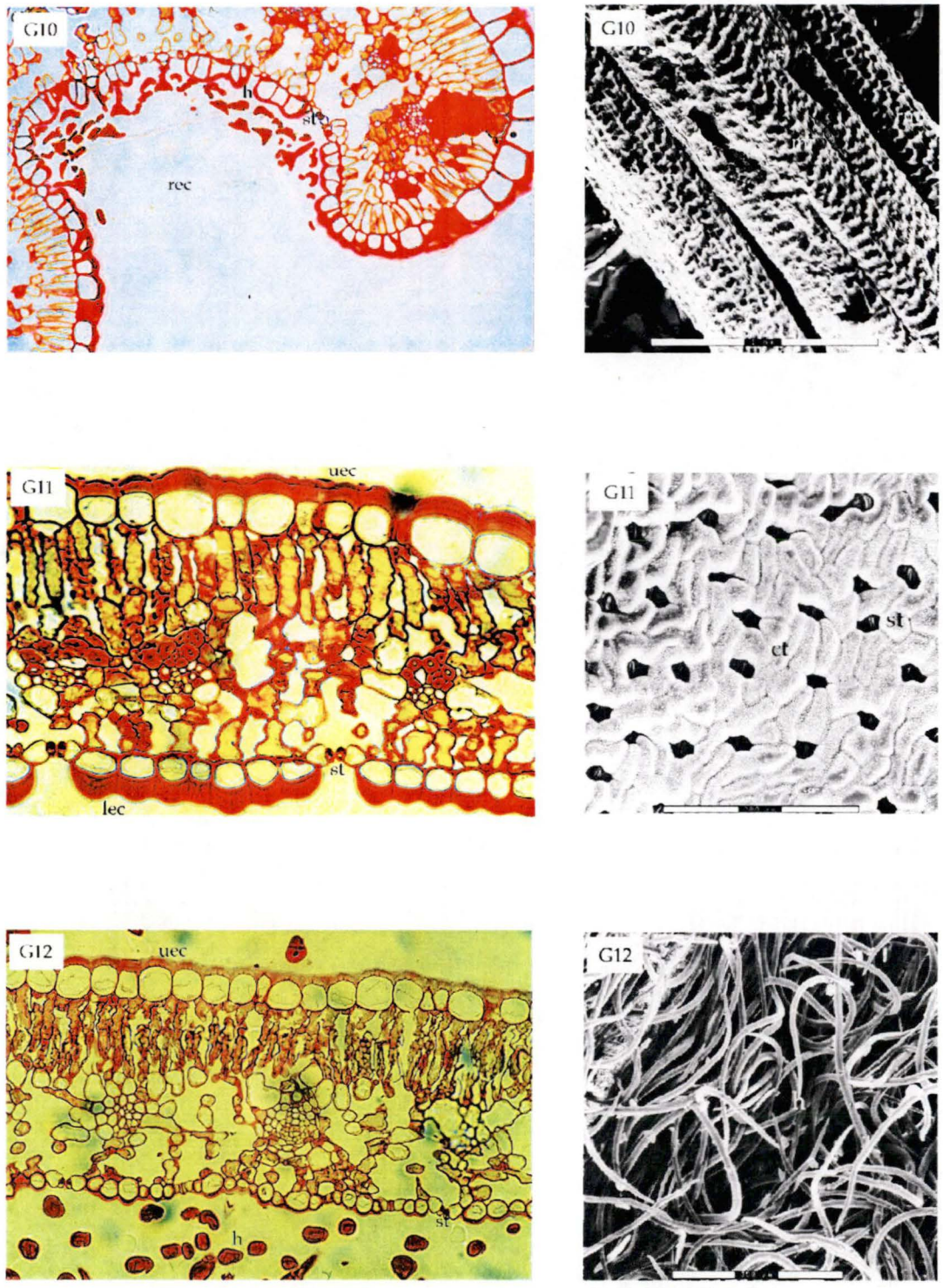


Figures2.20b. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *G. sericea* (G4-left, 360x), *G. lavandulacea* (G5-left, 320x), and *G. spinosissima* (G6-left, 320x). Note the presence of thick cuticle on the upper surface leaves, and stomates are covered by dense hairs found to be arranged parallel in *G. sericea* but dispersed in *G. lavandulacea*. Hairs in *G. spinosissima* are covered by leaf recurvations.

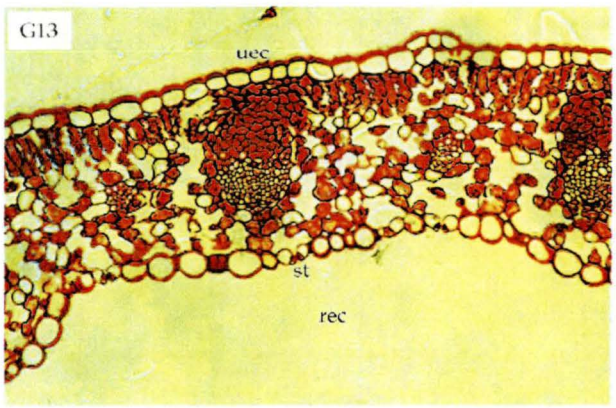
h = hairs; mr = midrib; rec = recurvation; rm = recurved margin; st = stomate; uec = upper epidermis cuticle.

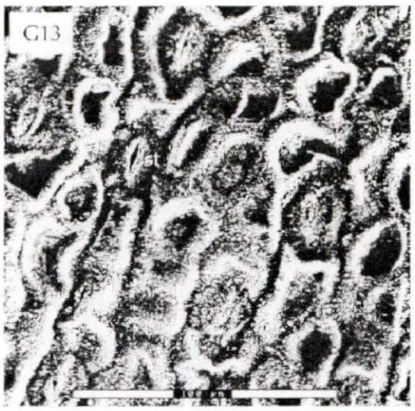


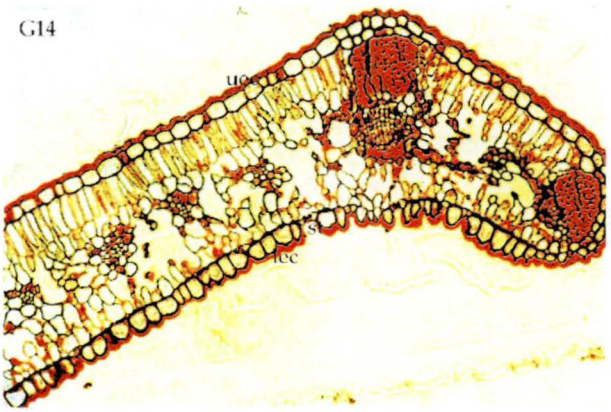
Figures2.20c. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *G. phanerophlebia* (G7-left, 160x), *G. pilosa* ssp. *pilosa*, (G8-left, 320x), and *G. lissopleura* (G9-left, 640x). Note, in *G. phanerophlebia* and *G. pilosa* ssp. *pilosa*, thick cuticles with epidermis projections together make up stomatal depressions. In these species hairs are not as dense as in the previous species, however in *G. lissopleura* dense hairs are very protected inside leaf recurvations.
h = hairs; hairs; lec = lower epidermis cuticle; mr = midrib; rec = recurvation; rm = recurved margin; st = stomate; uec = upper epidermis cuticle.

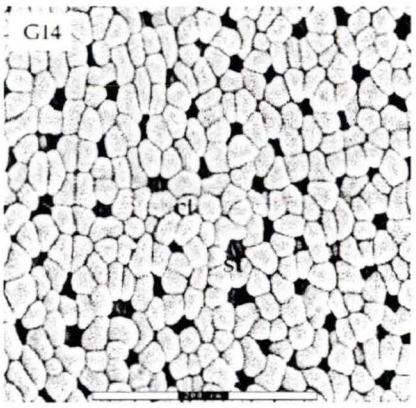


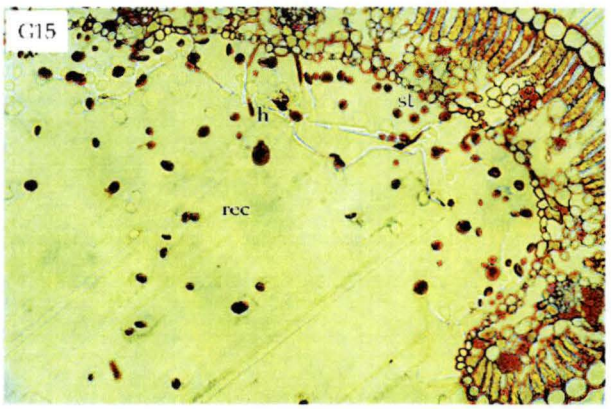
Figures2.20d. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *G. nudiflora* (G10-left, 160x), *G. bipinnatifida* (G11-left, 320x), and *G. beadleana* (G12-left, 160x). Note, that in *G. nudiflora* and *G. bipinnatifida,* epidermis projections together with thick cuticles form stomatal depressions. Hairs are few in the latter species, however in the former species they are dense and hidden inside the leaf recurvations. Dense hairs are also found in *G. beadleana*, and in this species stomates are on the surface.
h = hairs; lec = lower epidermis cuticle; mr = midrib; rec = recurvation; rm = recurved margin; st = stomate; uec = upper epidermis cuticles.

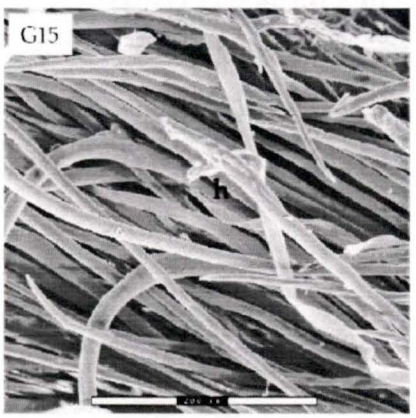




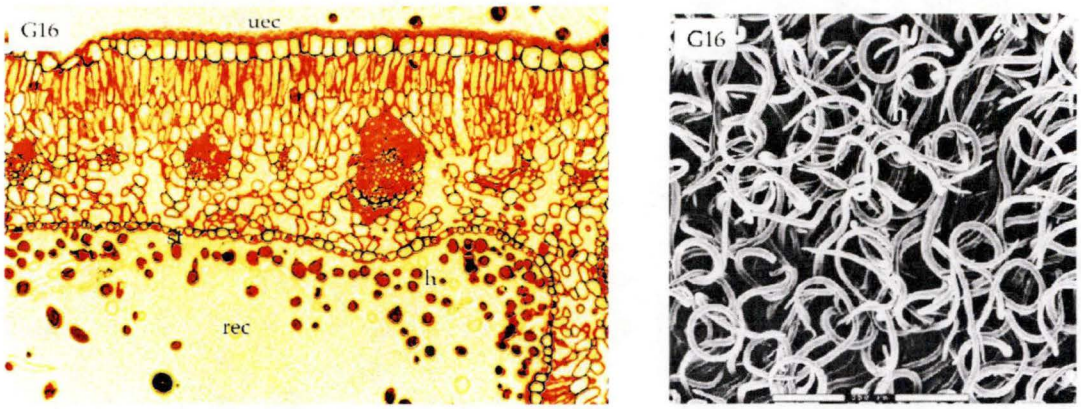








Figures 2.20e. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *G. iaspicula* (G13-left, 160x), *G. manglesii* ssp. *dissectifolia* (G14-left, 160x), and *G. caleyi* (G15-left, 160x). Leaves of *G. iaspicula* and *G. manglesii* ssp. *manglesii* are glabrous. The latter species has stomates in depressions which are formed from epidermis projections and thick cuticles on their surface. *G. iaspicula* has stomates on the surface, but they are protected by a parallel arrangement of dense hairs.
h = hairs; lec = lower epidermis cuticle; rec = recurvation; st = stomate; uec = upper epidermis cuticle



Figures2.20f. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *G. willisii* (G16-left,160x). In this species, stomates are protected by leaf recurvations and dense hairs.
h = hairs; rec = recurvation; st = stomate; uec = upper epidermis cuticle

1. Stomatal protection components of *Grevillea vs.* habitat rainfall

Table 2.10. Indices, cuticle thickness and habitat rainfalls for every *Grevillea* species observed.

Id = depression index
Irm = index of recurved margin
Ih = hair index ($x10^2\mu m^2$)
Ie = epidermis index
Sc = stomatal cuticle thickness (μm)
Uec = the thickness of the upper epidermis cuticle (μm)
Lec = the thickness of cuticle (μm)
Rf = minimum of the range of annual of the lower epidermis rainfall of habitat (mm)

Species	Id	Irm	Ih	Ie	Sc	Uec	Lec	Rf
G. johnsonii	3.80 ±0.13	3.08 ±0.53	5.30 ±0.22	1.07 ±0.17	0.38	5.25 ±0.60	0.90 ±0.02	800
G. aquifolium	3.39 ±0.16	1.00	2.54 ±0.09	0.59 ±0.03	0.88 ±0.03	9.7 ±0.45	1.97 ±0.06	375
G. aspera	1.00	1.13 ±0.01	16.16 ±0.91	1.11 ±0.03	0.75	23.13 ±0.45	2.13 ±0.10	200
G. sericea	3.73 ±0.53	1.13 ±0.01	1.70 ±0.05	0.67 ±0.03	0.38	6.90 ±0.45	2.40 ±0.08	500
G. lavandulacea	1.00	2.86 ±0.32	17.80 ±1.07	1.65 ±0.09	0.38	15.62 ±0.30	0.75	200
G. spinosissima	1.00	11.28 ±0.38	1.88 ±0.03	0.22 ±0.01	0.75	8.11 ±0.15	3.37 ±0.05	325
G. phanerophlebia	7.93 ±0.30	1.00	1.72 ±0.07	0.45 ±0.03	0.75	12.16 ±0.45	12.33 ±0.49	250
G. pilosa ssp. *pilosa*	24.76 ±1.97	1.00	3.36 ±0.16	0.84 ±0.01	0.75	12.02 ±0.75	16.07 ±0.97	250
G. lissopleura	1.00	5.60 ±0.46	4.14 ±0.26	0.42 ±0.02	0.38	23.25 ±0.60	2.01 ±0.14	300
G. nudiflora	7.60 ±1.02	2.15 ±0.16	3.34 ±0.24	0.82 ±0.01	.95	6.90 ±0.45	3 ±0.27	500
G. bipinnatifida	6.02 ±0.22	1.38 ±0.07	0.1 ±0.01	0.02	0.57	12.01 ±0.45	10.87 ±0.42	600
G. beadleana	1.00	1.38 ±0.07	11.95 ±0.32	0.71 ±0.03	0.38	8.26 ±0.75	1.89 ±0.08	800
G. iaspicula	1.00	1.49 ±0.02	0	0	0.75	7.20 ±0.15	0.75	600
G. manglesii ssp. *dissectifolia*	7.68 ±0.33	1.10 ±0.02	0	0	0.57	8.56 ±0.45	4.94 ±0.19	850
G. caleyi	1.00	1.79 ±0.11	28.17 ±1.38	0.80 ±0.04	0.38	2.85 ±0.45	0.38	1000
G. willisii	1.00	1.67 ±0.03	9.74 ±0.79	1.10 ±0.11	0.38	13.36 ±1.26	2.07 ±0.28	800

Figure 2.21. Graphs showing correlations between stomatal protection components of *Grevillea* species and minimum of the range of annual rainfall of the habitat.

A. Index depression *vs.* rainfall. The coefficient of correlation of -0.231 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs.* rainfall. The coefficient of correlation of 0.230 was found to be not significant ($p>0.1$).

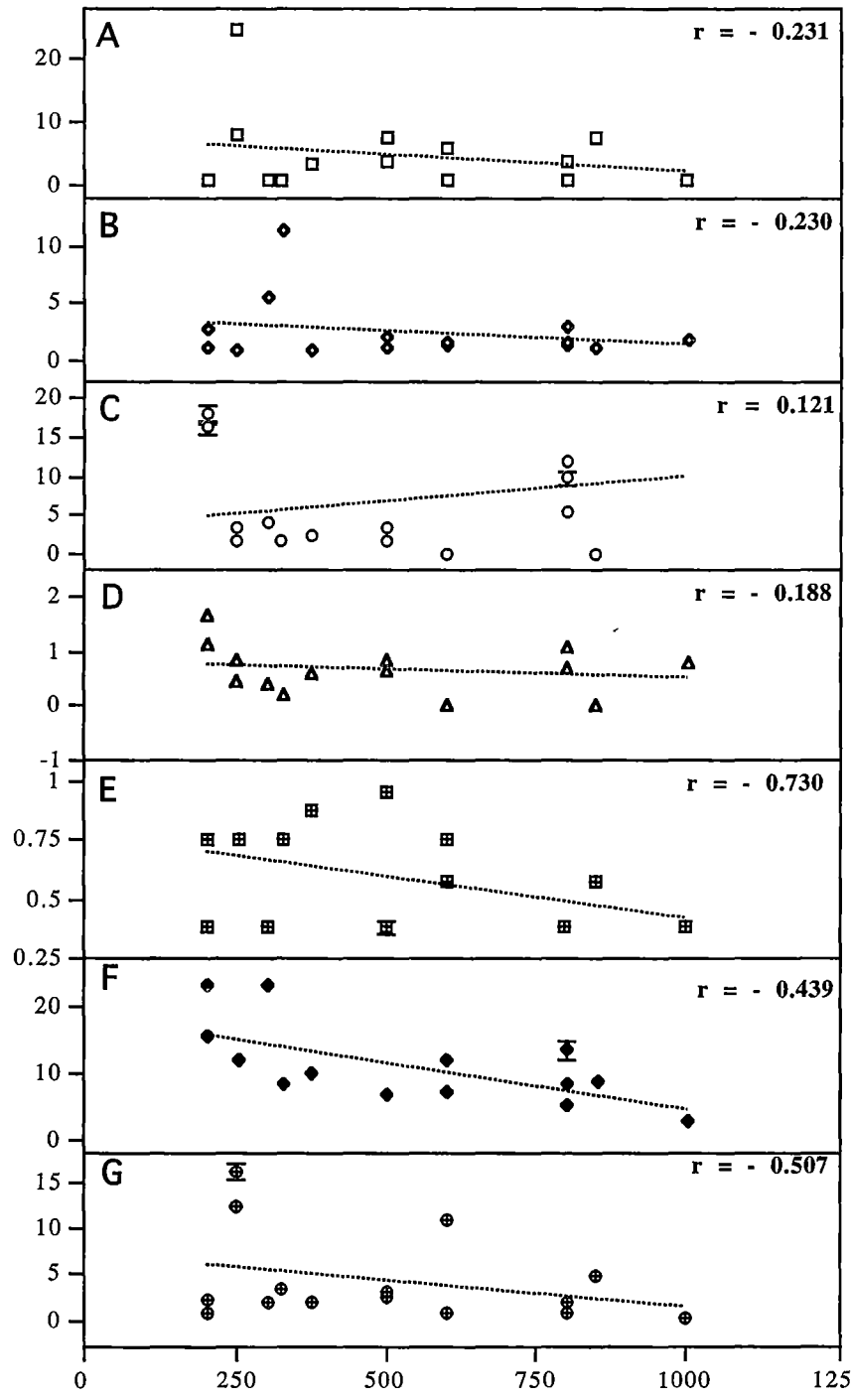
C. Index hair *vs.* rainfall. The coefficient of correlation of 0.121 was found to be not significant ($p>0.1$).

D. Index epidermis *vs.* rainfall. The coefficient of correlation of -0.188 was found to be not significant ($p>0.1$).

E. Stomatal cuticle *vs.* rainfall. The coefficient of correlation of -0.730 was found to be highly significant ($p<0.01$).

F. Upper epidermis cuticle *vs.* rainfall. The coefficient of correlation of -0.454 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* rainfall. The coefficient of correlation of -0.513 was found to be significant ($p<0.05$).


A
r = - 0.231
B
r = - 0.230
C
r = 0.121
D
r = - 0.188
E
r = - 0.730
F
r = - 0.439
G
r = - 0.507
0
250
500
750
1000
1250
Rainfall (mm)

These correlations represent all the species observed. Similar correlations with different sources and amounts of data will be presented, in order to obtain complete information about the association between characters observed and climatic factors of the habitat.

Stomatal depressions (r =-0.231), recurved margins (0.230), hair size (0.121), ratio of the number of hairs to number of stomates (-0.188) and the thickness of upper epidermis cuticle (-0.439) showed no correlation ($p>0.05$) with the minimum of the range of annual rainfall (Figure 2.21). This is an interesting finding since in the gross morphology recurved margins and hairs are prominent in this genus. The thickness of the stomatal (-0.730) and the lower epidermis cuticle (-0.507) revealed strong correlation ($p<0.005$ and $p<0.05$ respectively) with minimum of the range of annual rainfall (figure 2.21). These results support the hypothesis that cutin and wax layers are continuous and very hydrophobic, and support the epidermal layer in protecting its water content, water absorption and water containing material (Romberger *et al.*, 1992).

In general, regardless of the existence of an association between the minimum of the range of annual rainfall and morphological characters, the graphs showed a tendency to decline in almost every character value with reducing minimum rainfall. Hair index (0.120) displayed a different trend, and is opposite what is expected (Figure 2.21).

Stomatal and lower epidermis cuticle thicknesses showed negative correlations with minimum of the range of annual rainfall ($p<0.05$). Cuticle is reported to be important in reflecting high irradiance (Robinson, *et al.* 1993), and limiting transcuticular water and solute flows (Martin and Juniper, 1970; Romberger *et al.*, 1992). Other characters displayed negative trends with reducing minimum of the range of annual rainfalls, except hair index and index of recurved margin (Table 2.12). It is very likely that these characters have different functions from those previously assumed.

Hair index (0.054), epidermis index (0.033), and the upper (0.310) and lower epidermis cuticle thickness (0.315) showed positive trends with increasing maximum annual temperature. Depression index (-0.213) and the index of revolute margin (-0.230) and stomatal cuticle thickness (-0.730) displayed the opposite trends.

Table 2.11. The index component (IC) and cuticle component (CC) values and the ranges of habitat climatic factors (estimated by ESOCLIM analysis) for every *Grevillea* species observed.
Id = depression index; Irm = index of recurved margin (μm); Ih = hair index (x10²μm²); Ie = epidermis index ; Sc = stomatal cuticle thickness (μm) ; Uec = thickness of upper epidermis cuticle (μm) ; Lec =thickness of lower epidermis cuticle (μm); Rf = the range of annual rainfall (mm) ; T = the range of annual average temperature (°C) ; R = the range of daily radiation (Mj/m²/day) ; Wr = the range of daily wind run (km/day)

Species	IC				CC			Climatic factors			
	Id	Irm	Ih	Ie	Sc	Uec	Lec	Rf	T	R	Wr
G. johnsonii	3.80 ±0.13	3.08 ±0.53	5.30 ±0.22	1.07 ±0.17	0.38 ±0.00	5.25 ±0.60	0.90 ±0.02	558-749	6.60-23.80	16.70-17.50	301-312.80
G. aquifolium	3.39 ±0.16	0.00	2.54 ±0.09	0.59 ±0.03	0.88 ±0.03	9.70 ±0.45	1.97 ±0.06	505-1132	6.40-20.50	15.10-17.00	322-339
G. aspera	1.00	1.13 ±0.01	16.16 ±0.91	1.11 ±0.03	0.75	23.13 ±0.45	2.13 ±0.10	235-418	9.90-24.70	16.90-18.80	253- 288.70
G. sericea	3.73 ±0.53	1.13 ±0.01	1.70 ±0.05	0.67 ±0.03	0.38	6.90 ±0.45	2.40 ±0.08	550-1443	7-23.70	16.30-17.70	303- 311.60
G. lavandulacea	1.00	2.86 ±0.32	17.80 ±1.07	1.65 ±0.09	0.38	15.62 ±0.30	0.75	551-1127	7.20-21.60	15.50-17.60	326-382
G. nudiflora	6.02 ±0.22	2.15 ±0.16	3.34 ±0.24	0.82 ±0.01	0.95	6.90 ±0.45	3.00 ±0.27	392-637	9.30-22.50	17.40-18.20	314.40-317
G. bipinnatifida	7.60 ±1.02	1.38 ±0.07	0.10 ±0.01	0.02	0.57	12.01 ±0.45	10.87 ±0.42	416-1228	10-24.40	18.20-19.40	323 - 337
G. iaspicula	0.00	1.49 ±0.02	0	0	0.75	7.20 ±0.15	0.75	694-863	6.70-21.10	16.90-17	346-357.40
G. caleyi	0.00	1.79 ±0.11	28.17 ±1.38	0.80 ±0.04	0.38	2.85 ±0.45	0.38	1185-1351	11-22.60	16.60	323-337

Figure2.22. Graphs showing correlations between stomatal protection components of *Grevillea* species and estimated minimum of the range of annual rainfall of the habitat.

A. Index depression *vs.* rainfall. The coefficient of correlation of -0.407 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs.* rainfall. The coefficient of correlation of -0.141 was found to be not significant ($p>0.1$).

C. Index hair *vs.* rainfall. The coefficient of correlation of 0.401 was found to be not significant ($p>01$).

D. Index epidermis *vs.* rainfall. The coefficient of correlation of -0.321 was found to be not significant ($p>0.1$).

E. Stomatal cuticle *vs.* rainfall. The coefficient of correlation of -0.675 was found to be significant ($p<0.05$).

F. Upper epidermis cuticle *vs.* rainfall. The coefficient of correlation of -0.467 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* rainfall. The coefficient of correlation of -0.689 was found to be significant ($p<0.05$).

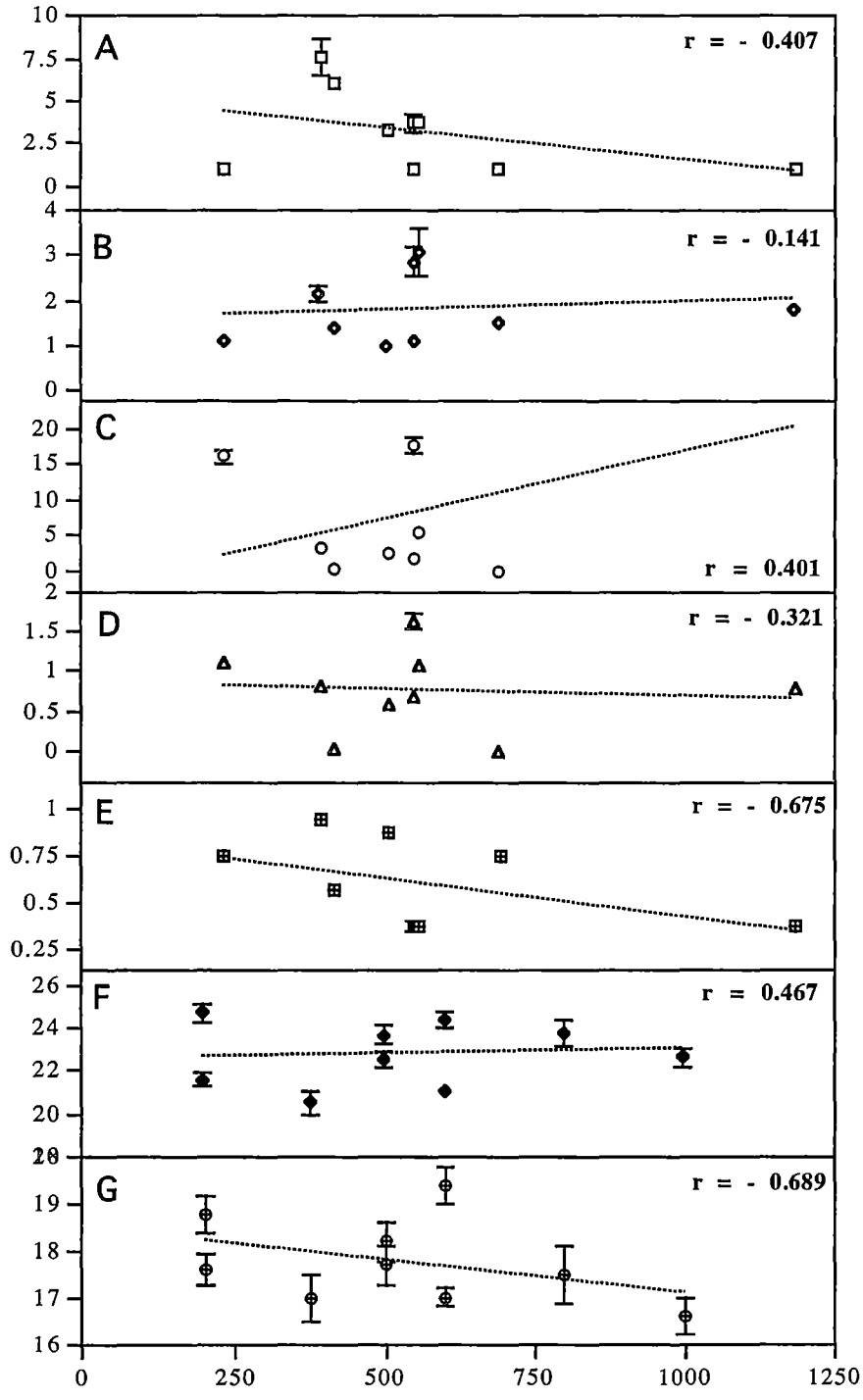

A
r = - 0.407
B
r = - 0.141
C
r = 0.401
D
r = - 0.321
E
r = - 0.675
F
r = 0.467
G
r = - 0.689
0
250
500
750
1000
1250
Rainfall (mm)

Figure2.23. Graphs showing correlations of stomatal protection components of *Grevillea* species and estimated maximum annual temperature of the habitat.

A. Index depression *vs.* temperature. The coefficient of correlation of 0.231 was found to be not significant ($p>0.1$)

B. Index recurved margin *vs.* temperature. The coefficient of correlation of 0.053 was found to be not significant ($p>0.1$).

C. Index hair *vs.* temperature. The coefficient of correlation of 0.054 was found to be not significant ($p>0.1$).

D. Index epidermis *vs.* temperature. The coefficient of correlation of 0.033 was found to be not significant ($p>0.1$).

E. Stomatal cuticle *vs.* temperature. The coefficient of correlation of -0.257 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle *vs.* temperature. The coefficient of correlation of 0.310 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* temperature. The coefficient of correlation of 0.315 was found to be not significant ($p>0.1$).

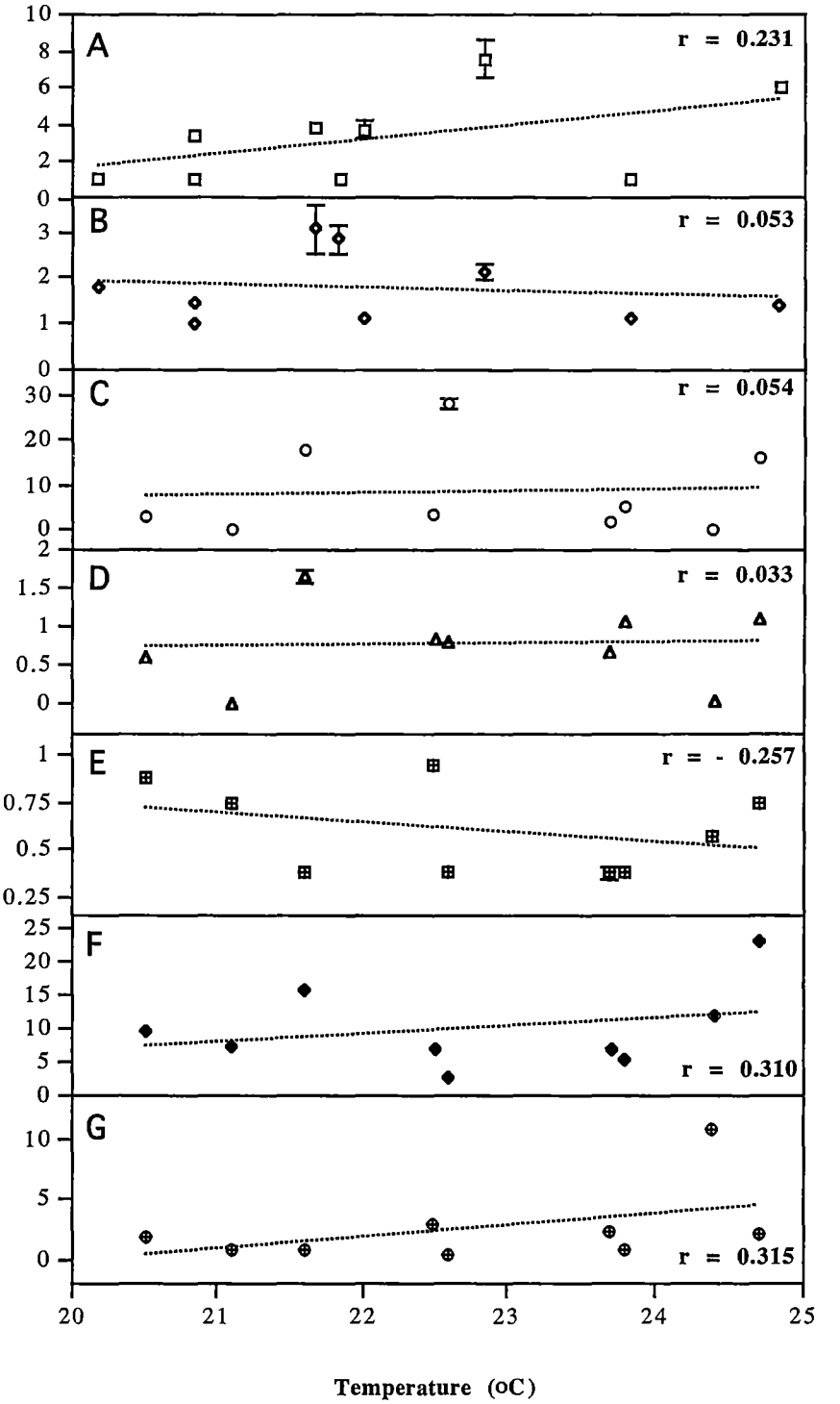

A
r = 0.231
B
r = 0.053
C
r = 0.054
D
r = 0.033
E
r = - 0.257
F
r = 0.310
G
r = 0.315
Temperature (oC)

Figure2.24. Graphs showing correlations of stomatal protection components of *Grevillea* species and estimated maximum of the range of daily radiation of the habitat.

A. Index depression *vs.* radiation. The coefficient of correlation of 0.493 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs.* radiation. The coefficient of correlation of -0.151 was found to be not significant ($p>0.1$).

C. Index hair *vs.* radiation. The coefficient of correlation of -0.280 was found to be not significant ($p>0.1$).

D. Index epidermis *vs.* radiation. The coefficient of correlation of -0.171 was found to be not significant ($p>0.1$).

E. Stomatal cuticle *vs.* radiation. The coefficient of correlation of 0.542 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle *vs.* radiation. The coefficient of correlation of 0.193 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* radiation. The coefficient of correlation of 0.806 was found to be significant ($p<0.1$).

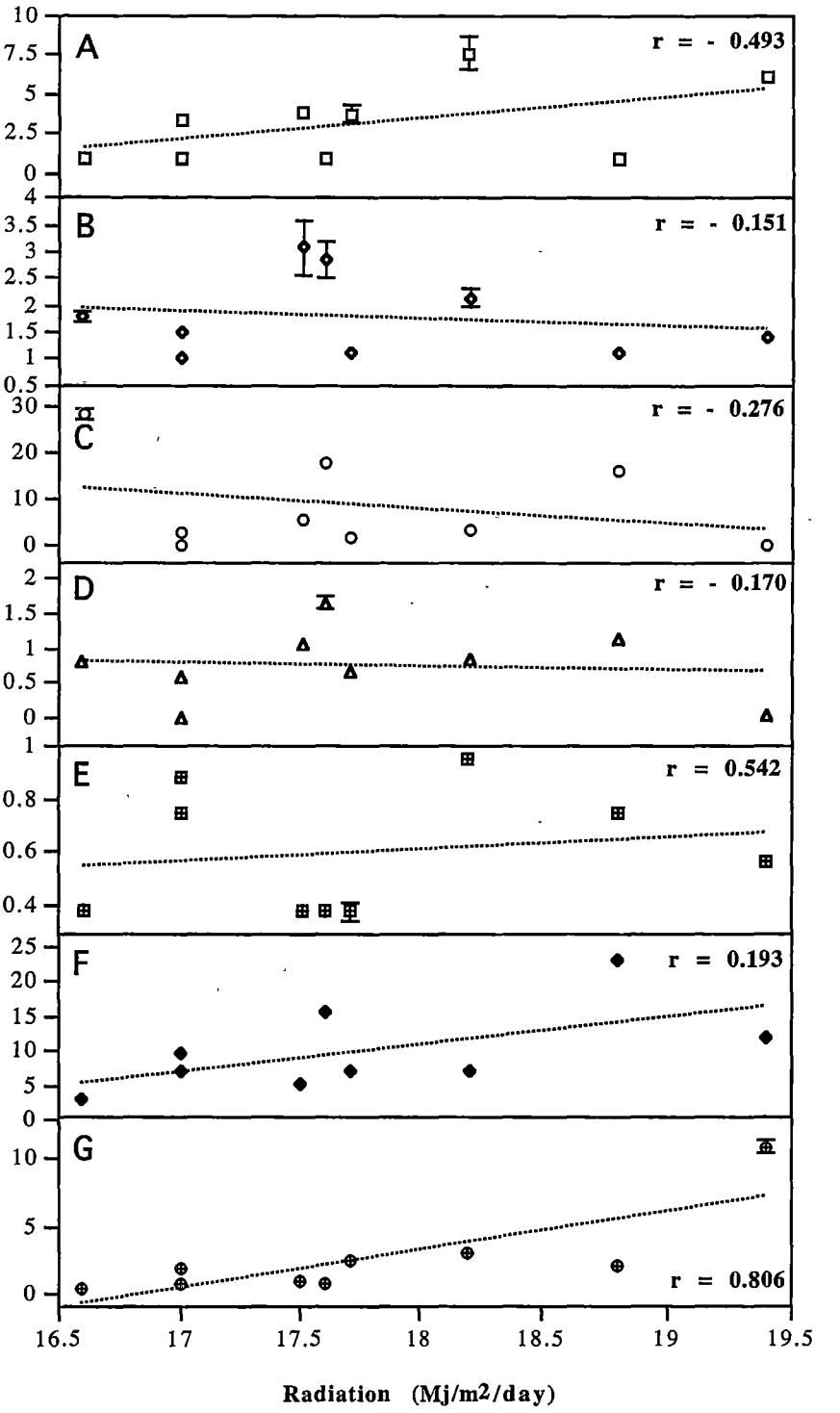

A
r = - 0.493
B
r = - 0.151
C
r = - 0.276
D
r = - 0.170
E
r = 0.542
F
r = 0.193
G
r = 0.806
16.5
17
17.5
18
18.5
19
19.5
Radiation (Mj/m2/day)

Figure2.25. Graphs showing correlations of stomatal protection components of *Grevillea* species and estimated maximum of the range of daily wind run of the habitat.

A. Index depression *vs.* wind run. The coefficient of correlation of -0.281 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs.* wind run. The coefficient of correlation of 0.327 was found to be not significant ($p>0.1$).

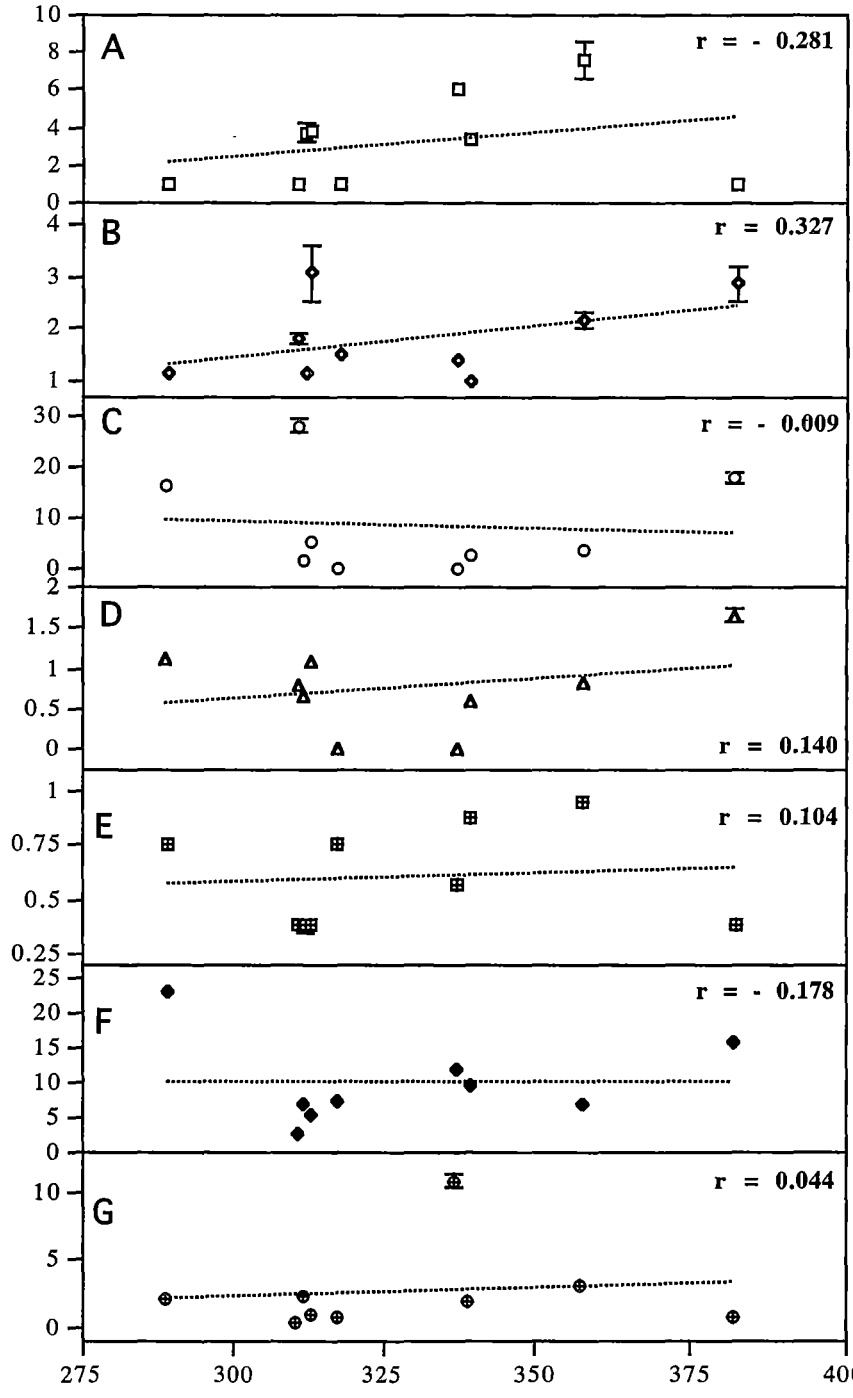
C. Index hair *vs.* wind run. The coefficient of correlation of -0.009 was found to be not significant ($p>0.1$).

D. Index epidermis *vs.* wind run. The coefficient of correlation of 0.140 was found to be not significant ($p>0.1$)

E. Stomatal cuticle *vs.* wind run. The coefficient of correlation of 0.104 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle *vs.* wind run. The coefficient of correlation of -0.178 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* wind run. The coefficient of correlation of 0.044 was found to be not significant ($p>0.1$).


A
r = - 0.281
B
r = 0.327
C
r = - 0.009
D
r = 0.140
E
r = 0.104
F
r = - 0.178
G
r = 0.044
275
300
325
350
375
400
Wind run (km/day)

In order to investigate which dimensions of the characters give large contributions to the character performances a correlation analysis between every dimension with the climatic factor was applied.

Table 2.12. Correlation (coefficient value and probability) between character dimensions of *Grevillea* species and climatic factors
Dim = dimension; Rft = minimum of the range of annual rainfall; Rf = estimated minimum of the range of annual rainfall; T = maximum of the range of annual temperature; R = maximum of the range of daily radiation; Wr = maximum of the range of daily wind run; c = count; r = correlation coefficient; p = probability; Id = depression index; dw = depression width; dd = depression depth; Irm = index of recurved margin; rs and rm = width of surface and median recurvation; rd = recurvation depth; Ih = hair index; vhl and vhd = the length and diameter of vertical hairs ; hhl and hhd = the length and diameter of horizontal hairs ; Ie = epidermis index; dd = hair density; ds = stomate density; Sc, Uec and Lec = the thickness of stomatal cuticle and upper and lower epidermis cuticle.

Dim	Rft			Rf			T		R		Wr	
	c	r	p	c	r	p	r	p	r	p	r	p
Id[1]	16	-0.163	>0.1	9	-0.350	>0.1	0.200	>0.1	0.284	>0.1	-0.381	>0.1
dw[1]	16	-0.075	>0.1	9	-0.355	>0.1	0.189	>0.1	0.271	>0.1	-0.359	>0.1
dd[1]	16	-0.094	>0.1	9	-0.394	>0.1	0.204	>0.1	0.377	>0.1	-0.338	>0.1
Irm[1*]	16	0.327	>0.1	9	0.323	>0.1	0.048	>0.1	-0.241	>0.1	0.024	>0.1
Ih[2]	16	0.121	>0.1	9	0.401	>0.1	0.054	>0.1	-0.276	>0.1	0.008	>0.1
vhl[2]	16	0.114	>0.1	9	-0.075	>0.1	0.491	>0.1	0.347	>0.1	-0.524	>0.1
vhd[2]	16	-0.460	>0.1	9	-0.338	>0.1	0.502	>0.1	0.508	>0.1	-0.444	>0.1
hhl[2]	16	0.012	>0.1	9	0.293	>0.1	0.281	>0.1	-0.012	>0.1	-0.110	>0.1
hhd[2]	16	-0.201	>0.1	9	-0.064	>0.1	0.434	>0.1	0.269	>0.1	-0.464	>0.1
Ie[2]	16	-0.118	>0.1	9	-0.025	>0.1	0.032	>0.1	0.170	>0.1	-0.138	>0.1
ds[2]	16	-0.038	>0.1	9	0.070	>0.1	-0.099	>0.1	-0.052	>0.1	0.457	>0.1
dh[2]	16	-0.219	>0.1	9	-0.031	>0.1	0.015	>0.1	0.113	>0.1	0.022	>0.1
Sc[3]	16	-0.730	<0.005	9	-0.676	<0.05	0.256	>0.1	0.542	>0.1	-0.103	>0.1
Uec[1]	16	-0.439	>0.05	9	-0.471	>0.1	-0.309	>0.1	0.193	>0.1	-0.173	>0.1
Lec[1]	16	-0.507	<0.05	9	-0.688	<0.05	0.325	>0.1	0.806	<0.05	0.046	>0.1

[1]-transformation - $\log_{10}(Y+1)$
[2]-transformation - $\sqrt{Y}$
[3]-untransformed
*-have relative values

In response to increasing maximum daily radiation, depression index (0.231), thickness of stomatal cuticle (0.542), and the upper (0.193) and lower (0.806) epidermis cuticle thickness showed positive trends. Among these characters the correlation coefficient of the lower epidermis cuticle thickness was significant ($p<0.05$). There is a possibility that this character has an important role in reflecting incoming radiation. The opposite trend was displayed by the recurved margin (-0.240), hair (-0.280) and epidermis index (-0.171).

The index of recurved margin (0.327), epidermis (0.140), thickness of stomatal (0.104) and the lower epidermis cuticle (0.044) showed positive trends with increasing maximum daily wind run. Negative trends were displayed by depression (-0.281), hair index (-0.009) and the thickness of the upper epidermis cuticle (-0.178). Among characters analysed, depression index and the index of recurved margin showed the strongest trend. This means that there is the possibility that stomatal depressions and recurved margins could securely protect stomates from the breeze which can sweep away water vapour.

Analysis of variance for the characters measured (Table 2.13) was applied. The result shows that there are highly significant differences in the value of every index calculated among species observed (Figure 2.26). These graphs provide a clearer description of structural diversity of stomatal protection components in leaves of *Grevillea* species.

Table 2.13. Significance levels of every index calculated and cuticle thickness measured among *Grevillea* species observed.

Indices and cuticle thickness	F-test	Prob.	Transform.
Depression index (Id)	73.31	0.0001	$\log_{10}(y+1)$
Index of recurved margin (Irm)	77.44	0.0001	$\log_{10}(y+1)$
Hair index (Ih)	343.25	0.0001	$\sqrt{y}$
Epidermis index (Ie)	179.67	0.0001	$\sqrt{y}$
Stomatal cuticle (Sc)*	-	-	-
Upper epidermis cuticle (Uec)	58.51	0.0001	untrans.
Lower epidermis cuticle (Lec)	425.76	0.0001	$\log_{10} y$

*not analysed due to no variation in observations

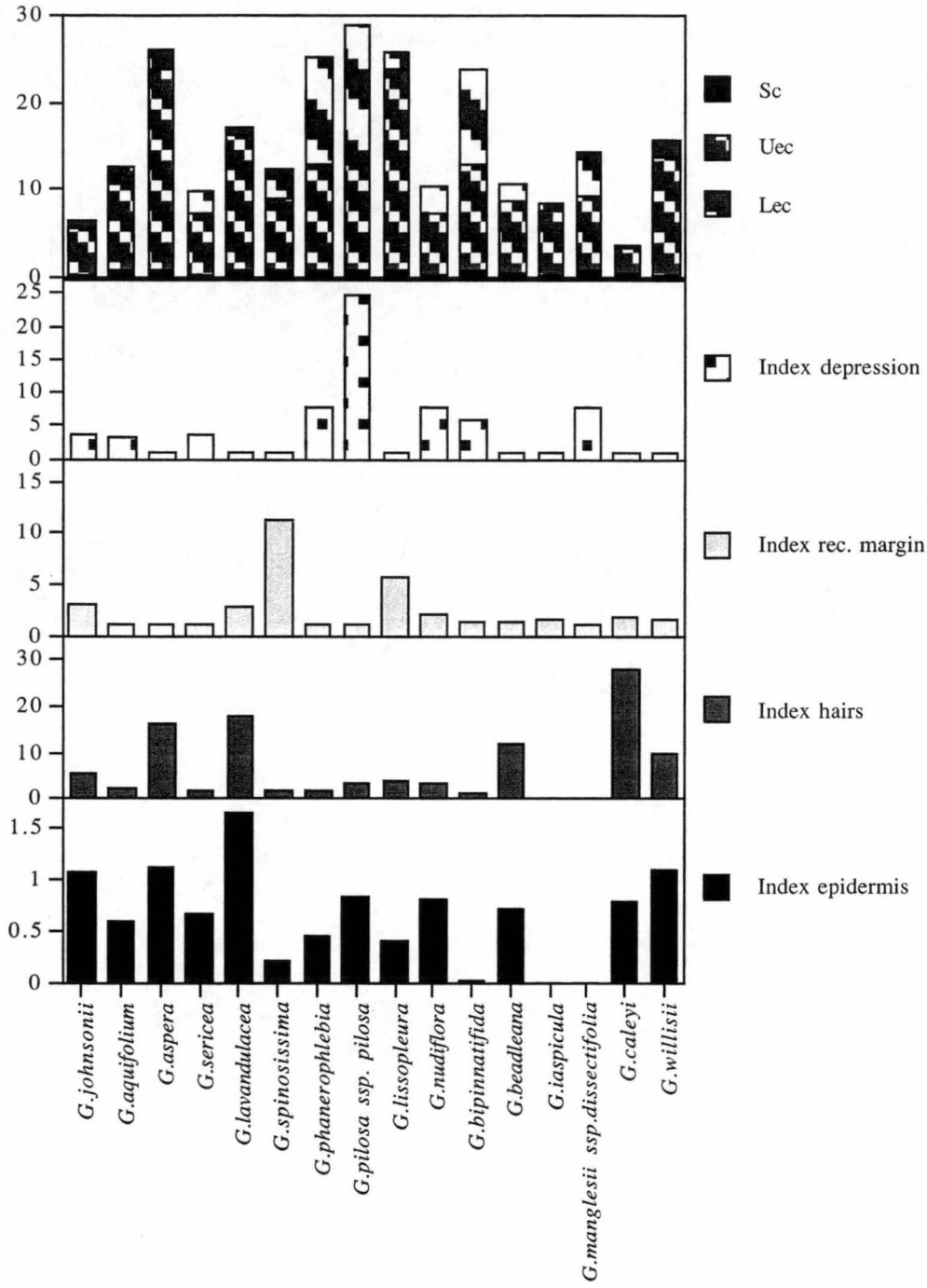


Figure 2.26. Graphs of index values and cuticle thickness for every species observed. These graphs clearly show the varieties and values of stomatal protection components in leaves of *Grevillea* species.
Id = depression index; Irm = index of recurved margin (Id and Irm - value 1 is equal to 0, see pages 39 - 40); Ih= hair index ($10^2\mu m^2$); Ie = epidermis index; Sc = stomatal cuticle thickness (μm); Uec = upper epidermis cuticle (μm); lec = lower epidermis cuticle (μm).

Tables 2.14a-g. Comparisons of mean values among *Grevillea* species observed in every index calculated. These tables describe the variety of the values of every index calculated among species observed

a. Depression index (Id)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
G. pilosa ssp. *pilosa*	5	24.477	A
G. phanerophlebia	5	7.913	B
G. manglesii ssp dissectifolia	5	7.654	B
G. nudiflora	5	7.366	B
G. bipinnatifida	5	6.002	B
G. johnsonii	5	3.794	C
G. sericea	5	3.624	C
G. aquifolium	5	3.384	C
*G. lissopleura**	5	1.000	
*G. aspera**	5	1.000	
*G. beadleana**	5	1000	
*G. lavandulacea**	5	1.000	
*G. caleyi**	5	1.000	
*G. iaspicula**	5	1.000	
*G. spinosissima**	5	1.000	
*G. willisii**	5	1.000	

b. Index of recurved margin (Irm)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
G. spinosissima	5	11.225	A
G. lissopleura	5	5.462	A
G. johnsonii	5	2.734	B
G. lavandulacea	5	2.719	B
G. nudiflora	5	2.099	B C
G. caleyi	5	1.756	C D
G. willisii	5	1.665	C D E
G. iaspicula	5	1.487	D E
G. bipinnatifida	5	1.367	E
G. beadleana	5	1.367	E
G. aspera	5	1.126	F
G. sericea	5	1.126	F
G. manglesii ssp. *dissectifolia*	5	1.103	F
G. phanerophlebia	5	1.000	
*G. aquifolium**	5	1.000	
G. pilosa ssp *pilosa**	5	1.000	

c. Hair index (Ih)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
G. caleyi	5	28.103	A
G. lavandulacea	5	17.729	B
G. aspera	5	16.107	B
G. beadleana	5	11.938	C
G. willisii	5	9.682	C
G. johnsonii	5	5.287	D
G. lissopleura	5	4.123	D E
G. pilosa ssp. pilosa	5	3.353	E F
G. nudiflora	5	3.325	E F
G. aquifolium	5	2.541	F G
G. spinosissima	5	1.877	G
G. phanerophlebia	5	1.719	G
G. sericea	5	1.696	G
G. bipinnatifida	5	0.096	H
G. manglesii ssp. *dissectifolia**	5	0.000	
*G. iaspicula**	5	0.000	

*not included in the analysis

d. Epidermis index (Ie)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
G. lavandulacea	5	1.642	A
G. aspera	5	1.112	B
G.willisii	5	1.088	B
G. johnsonii	5	1.072	B
G. pilosa ssp. pilosa	5	0.832	C
G. nudiflora	5	0.824	C
G. caleyi	5	0.800	C
G. beadleana	5	0.705	C D
G. sericea	5	0.667	C D
G. aquifolium	5	0.591	D E
G. phanerophlebia	5	0.444	E F
G. lissopleura	5	0.423	F
G. spinosissima	5	0.215	G
G. bipinnatifida	5	0.016	H
G. manglesii ssp.*dissectifolia**	5	0.000	
*G. iaspicula**	5	0.000	

* not included in the analysis

e. Stomatal cuticle (Sc)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
G. aspera	5	23.102	A
G. lissopleura	5	22.996	A
G. lavandulacea	5	15.594	B
G. willisii	5	13.294	B C
G. phanerophlebia	5	12.106	C D
G. pilosa ssp. *pilosa*	5	12.024	C D
G. bipinnatifida	5	11.942	C D
G. aquifolium	5	9.742	D E
G. manglesii ssp. *dissectifolia*	5	8.622	E
G. beadleana	5	8.210	E
G. spinosissima	5	8.172	E
G. iaspicula	5	7.268	E F
G. nudiflora	5	6.934	E F
G. sericea	5	6.934	E F
G. johnsonii	5	5.206	F G
G. caleyi	5	2.812	G

f. Upper epidermis cuticle

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
G. pilosa ssp. *pilosa*	5	15.899	A
G. phanerophlebia	5	12.301	B
G. bipinnatifida	5	10.848	B
G. manglesii ssp *dissectifolia*	5	4.930	C
G. spinosissima	5	3.377	D
G. lavandulacea	5	3.377	D
G. nudiflora	5	2.959	D E
G. sericea	5	2.404	E F
G. aspera	5	2.131	F
G. willisii	5	2.008	F
G. lissopleura	5	2.006	F
G. aquifolium	5	1.971	F
G. beadleana	5	1.890	F
G. johnsonii	5	0.904	G
G. iaspicula	5	0.750	G
G. caleyi	5	0.380	H

g. Lower epidermis cuticle (Lec)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
G. nudiflora	5	0.950	
G. aquifolium	5	0.900	
G. aspera	5	0.750	
G. phanerophlebia	5	0.750	
G. pilosa ssp. *pilosa*	5	0.750	
G. iaspicula	5	0.750	
G. spinosissima	5	0.750	
G. bipinnatifida	5	0.570	
G.manglesii ssp. *dissectifolia*	5	0.570	
G. lissopleura	5	0.380	
G. beadleana	5	0.380	
G. lavandulacea	5	0.380	
G. caleyi	5	0.380	
G. sericea	5	0.380	
G. johnsonii	5	0.380	
G. willisii	5	0.380	

* not analysed because variation within samples cannot be detected

Table 2.14 a shows that *G. pilosa* ssp. *pilosa* exhibited high depression index values. However, it appeared that stomatal depressions in *Grevillea* were not as protective as in *Banksia,* since they were only bordered by a one-celled layer. *G. johnsonii* and *G. aquifolium* had low Id values which did not show significant differences between them. Fifty percent of the species observed did not exhibit this structure (*e.g. G. aspera* and *G. lavandulacea*). It is possible that they possess other structures which are more protective than the given structure. Another possibility is climatic stress; the amount of rainfall, radiation and wind run of habitats does not stimulate plants to produce such a structure. In general, statistical analysis revealed no correlation ($p>0.1$) between depression index and any habitat climatic factor.

Recurved margins were commonly found in *Grevillea* leaves, however, they varied from recurved into revolute (Table 2.14 b). *G spinosissima* showed extreme revolute margins, that almost enclosed the undersurface and touched the midvein. Such a structure presumably has a big role in protecting the stomates from excessive transpiration. It is supported by the fact that this species grows in habitats with a minimum of the range of annual rainfall of 325 mm. Although some species showed a low index of recurved margins (*e.g. G. sericea* and *G. phanerophlebia*), the rest showed prominent recurvations (*e.g. G. johnsonii* and *G. iaspicula*). Species with prominent recurvations live in various rainfall ranges, with the amount varying from 200 to 1000 mm per year. The desert species, however, exhibited deeper recurvations (*e.g. G. lavandulacea*). This is possibly associated with the harsh climate that develops in a desert. Although some species seemed to exhibit associations with their habitat rainfall, statistical analysis did not show any association between them.

Most of the species observed have dense indumentums, except *G. iaspicula* and *G. manglesii* ssp. *dissectifolia* which did not possess hairs at all (Table 2.14c) Species with low levels of hair index inhabited places having a minimum annual rainfall varying from 250 - 800 mm. The ones with medium levels of hair index grew in areas with minimum annual rainfall ranging from 200 - 800 mm. However, species with a high level of hair index value i.e. *G. caleyi* lived in an area with a minimum range of annual rainfall of 1000 mm. Statistically, however, this phenomenon showed no correlation between minimum of the range of annual rainfall and hair index ($p>0.1$).

G. lavandulacea demonstrated a high value for epidermis index (Table 2.14d). It inhabits areas with a minimum of the range of annual rainfall of 200 mm. Species with low and medium level of epidermis indices inhabited areas with a wide range of rainfall, from 250 - 1000 mm. They did not exhibit patterns describing correlations between epidermis index and rainfall or other habitat climatic factors. Presumably, they may have other structures taking over the function of the characters that contribute to the epidermis index (trichomes). In this genus, cuticles and stomatal depressions may take that role.

It is assumed that the guard cell and subsidiary cell are mainly responsible for the amount of water evaporated. This is in accordance with Cowan's (1977) finding that guard cells and subsidiary cells are responsible for 77% of the total evaporation (Table 2.14e). The results here accord with this idea, since the correlation coefficient of stomatal cuticles with minimum of the range of annual rainfall were highly significant ($p<0.005$). *G. aspera* and *G. lissopleura,* which exhibited a high level of stomatal cuticle thickness, tended (Table 2.14e) to inhabit areas with minimum of the range of annual rainfall ranging from 200 - 300 mm. Others (*e.g. G. johnsonii, G. sericea, G. aquifolium*) with thinner stomatal cuticles tended to inhabit wetter areas.

The upper epidermis cuticle thickness seemed to be important for leaves of *Grevillea* species. This structure revealed strong correlations with almost every habitat climatic factor observed, except with maximum of the range of daily wind run (Table 2.12). Species with thin upper epidermis cuticle (*e.g. G. caleyi, G. aquifolium*)(Table 2.14f) inhabited areas with minimum of the range of annual rainfall of 200 - 1000 mm. *G. pilosa* ssp. *pilosa* which had thickest upper epidermis cuticle, tended to grow in areas with minimum of the range of annual rainfall of 250 mm.

G. nudiflora surpassed others in respect to the thickness of lower epidermis cuticle (Table 2.14g), and this species inhabited areas with a minimum of the range of annual rainfall of 500 mm. Species with the thinnest upper epidermis cuticle (*e.g. G. johnsonii, G. sericea*) tended to inhabit areas with minimum of the range of annual rainfall ranging from 200 - 1000 mm.

2.3.3. *Orites*

A. Leaf Morphology

Orites is a small genus with eight species (Wrigley and Fagg, 1989). However, they have many different leaf shapes, from needle (*O. acicularis*-Figure 2.26a-O1), to revolute (*O. revoluta*-Figure 2.26a-O2) to flat bifacial leaves (*O. diversifolia*-Figure 2.26b-O4). Unlike the two other genera, *Banksia* and *Grevillea,* which have dense leaf hairs, *Orites* has prominent cuticles covering the stomatal and epidermis cells (*e.g. O. milliganii*-Figure 2.26b-O6). The exception is *O. revoluta,* which has revolute margined leaves with very dense hairs on the lower surface. Hairs are also found on the leaves of *O. excelsa.* The role of these structures in relation to these species existence in their habitat will be discussed in detail later.

Morphological studies were conducted by observing the cross sections of leaves and ESEM images of the lower surface of leaves of *Orites* species (Figures 2.26 a, b-right).

B. 1. Correlation.

Correlation between components of stomatal protection and climatic factors of the habitat was investigated with the purpose of predicting the tendency for correlation. Due to the lack of published information about climatic factors of *Orites* habitats, an attempt was made to estimate the climatic factors from herbarium locality data, using ESOCLIM analysis. Then the estimated climatic factors were correlated with every character observed.

Figure 2.27 describes the association between characters assumed to increase the boundary layer against the minimum of the range of annual rainfall of *Orites* habitats. It seems that the characters assumed to increase the boundary layer did not show any correlation with the minimum of the range of annual rainfall of the habitats. It is

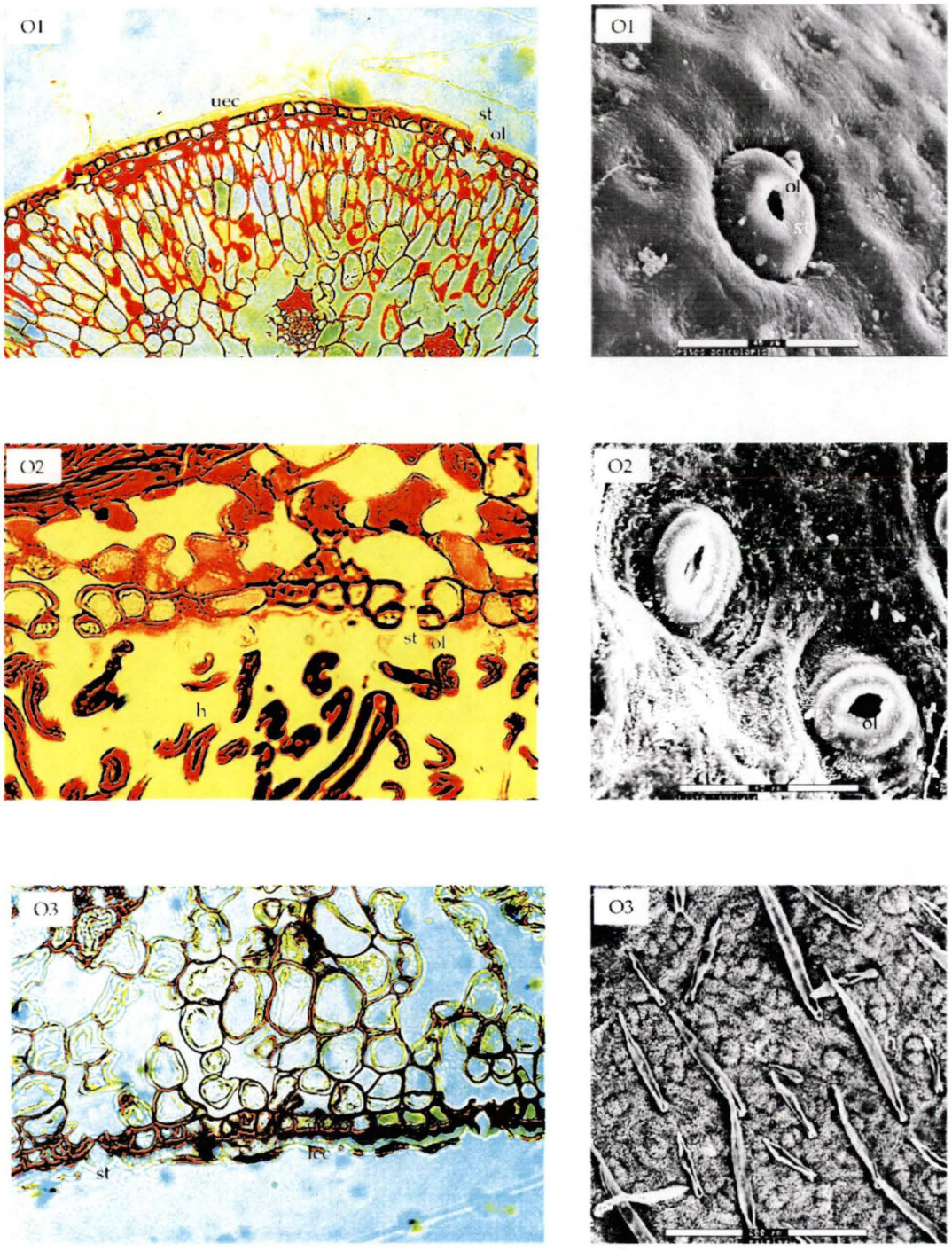
possible that water is not the limiting factor for these species. Another possibility is that there are other structures that indirectly protect stomates from excessive transpiration.

Characters that showed strong correlations with the maximum annual temperature of the habitats (Figures 2.28) were hair size ($r = 0.965$; $p<0.005$) and the ratio of hair number to stomate number ($r = 0.965$; $p<0.005$). However, it seems that the correlation coefficient does not show the real values, because of the lack of data provided. The scatter graphs produced only correlated the minimum and maximum values, without any other values scattered in between. Despite the accuracy of the correlations predicted, there is a possibility that these characters play roles in protecting stomates from incoming heat. This is in accordance with Wolpert's (1962) hypothesis that living hairs might function as heat conductor that could move heat away from the leaf surface.

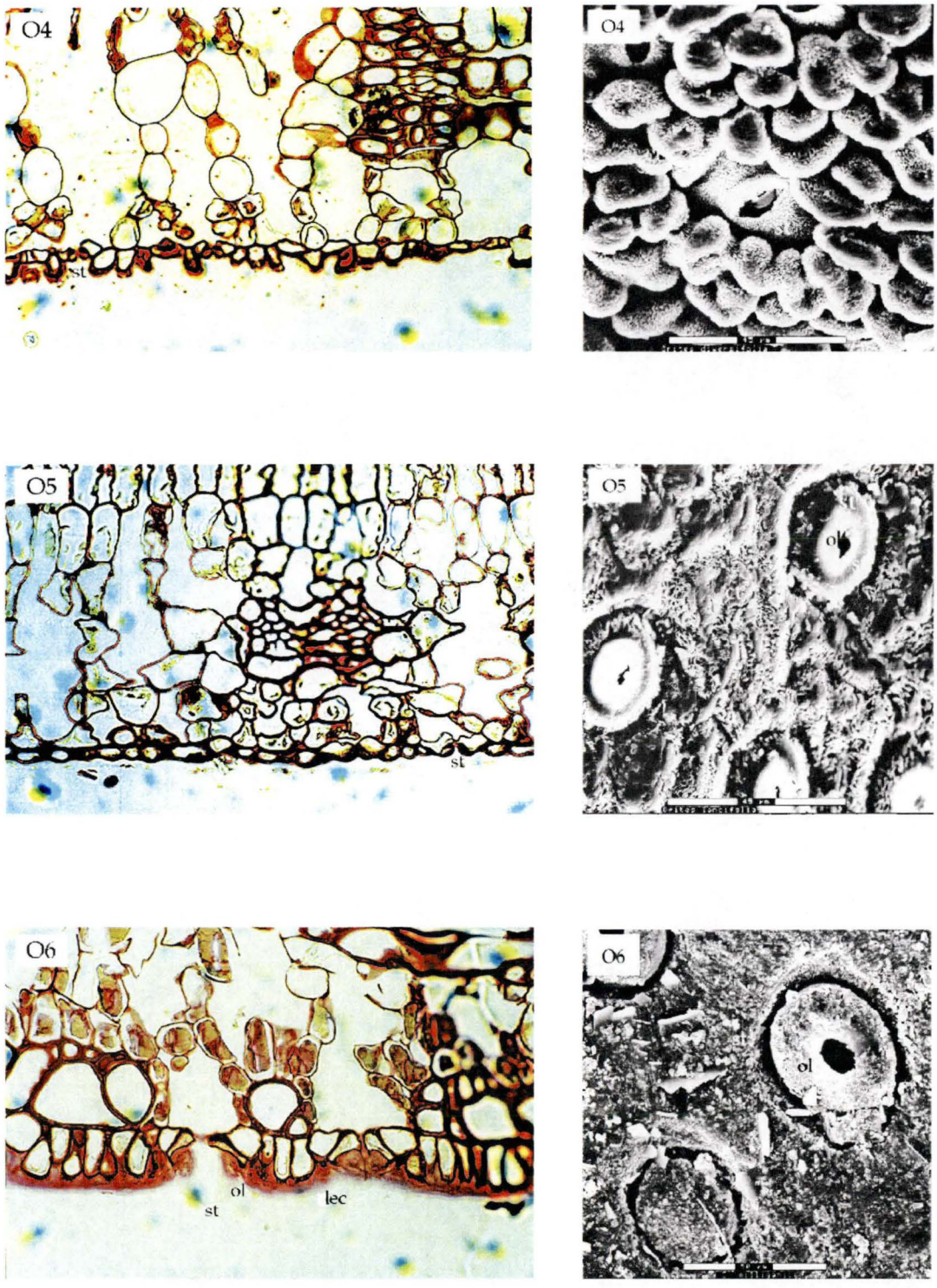
The density and dimensions of hairs provide good shelter for stomates and the epidermis (Wuencher, 1970). Moreover, glossy hair surfaces might reflect incoming radiation and thus reduce the absorption of heat and unnecessary light. Hairs take another role in stomatal protection, as was shown by strong correlations between hair size (0.965) and the ratio of hair number to stomate number (0.965) with maximum daily radiation (Figures 2.29). Hairs physically cover the stomates, therefore the water vapour cannot be swept away from the stomatal surface (Duddington, 1969).

So far, it is obvious that hairs play a large role in plant adaptation to habitat factors. Presumably, in *Orites* this structure is efficient at protecting the stomates from a harsh environment. This assumption is supported by the level of leaf plasticity in *O. revoluta* (Table 2.17).

In order to investigate which dimensions of the characters give large contributions to the given character performances, a correlation analysis between every dimension measured or calculated with the climatic factor was applied.



Figures2.26a. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *Orites acicularis* (O1-left, 200x), *O. revoluta* (O2-left, 400x), and *O. excelsa* (O3-left, 400x). In *O. acicularis*, stomates are in depressions formed by epidermis cells and thick cuticles. In *O. revoluta*, stomates are at the same level as the epidermis, yet, the outer ledges grow extensively. Dense hairs and revolute margin are also found in this species. *O. excelsa* has stomates with cuticle projection in the same level as the epidermis cuticle, and short hairs.
h = hairs; ol = outer ledges; rec = recurvation; st = stomate; uec = upper epidermis cuticle.



Figures2.26 b. Showing leaf cross sections and ESEM micrographs of the lower surface of leaves of *O. diversifolia* (O4-left, 200x), *O. lancifolia* (O5-left, 200-x), *O. milliganii* (O6-left, 400x). *O. diversifolia* has an interesting cuticle appearance, which looks like papillae with stomate sunken among them. *O. lancifolia* has extensive outer ledges that forming structures like stomatal lids. *O. milliganii* has stomates at the same level as the epidermis, however they have extensive outer ledges as in *O. lancifolia.* None of these species possess hairs.
lec = lower epidermis cuticle; ol = outer ledges; st = stomate; ct = cuticles.

Table 2.15. Index component (IC) and cuticle component (CC) values and the ranges of habitat climatic factors (estimated by ESOCLIM analysis) for every *Orites* species observed.
Id = depression index ; Irm = index of recurved margin ; Ih = hair index (μm^2); Ie = epidermis index; Sc = stomatal cuticle thickness (μm) ; Uec = thickness of upper epidermis cuticle(μm) ; Lec =thickness of lower epidermis cuticle (μm); Rf = the range of annual rainfall (mm) ; T = the range of annual average temperature (°C) ; R = the range of radiation ($Mj/m^2/day$); Wr = the range of wind run (km/day).

Species	IC				CC			Climatic factors			
	Id	Irm	Ih	Ie	Sc	Uec	Lec	Rf	T	R	Wr
O. acicularis	0	0	0	0	18.68 ±0.50	13.06 ±1.05	0	1018-2983	-1-14.70	12.30 -14.80	232.30-266.70
O. revoluta	0	3.00 ±0.50	0	0	3.60 ±0.27	3.15 ±0.30	2.30 ±0.09	842-2819	3-14.10	12.30-14.80	241.40-271.80
O. excelsa	0	0	185 ±4	0.44 ±0.01	12.75 ±0.68	10.21 ±0.15	10.18 ±0.21	716-7095	7.70-24.40	18-19.60	285.20-311.40
O. diversifolia	4.42 ±0.37	0	0	0	1.20 ±0.13	2.10 ±0.15	3.99 ±0.12	750-3292	0-15.90	12.30-14.80	233.90-273.40
O. lancifolia	0	0	0	0	5.48 ±0.23	1.50 ±0.15	3.80 ±0.12	1273-2452	-1-12.50	15.30-16.80	290.80-321.40
O. milliganii	0	0	0	0	15.6 ±0.63	6.75 ±0.75	7.22 ±0.33	1094-3325	0.40-14.80	12.10-14.70	246.40-275.10

Figure2.27. Graphs showing correlations of stomatal protection components of *Orites* species and estimated minimum of the range of annual rainfall of the habitat.

A. Index depression *vs*. rainfall. The correlation coefficient -0.448 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs*. rainfall. The correlation coefficient -0.241 was found to be not significant ($p>0.1$).

C. Index hair *vs*. rainfall. The correlation coefficient - 0.525 was found to be not significant ($p>0.1$).

D. Index epidermis *vs*. rainfall. The correlation coefficient -0.525 was found to be not significant ($p>0.1$).

E. Stomatal cuticle *vs*. rainfall. The correlation coefficient 0.240 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle *vs* rainfall. The correlation coefficient -0.257 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs*. rainfall. The correlation coefficient -0.230 was found to be not significant ($p>0.05$).

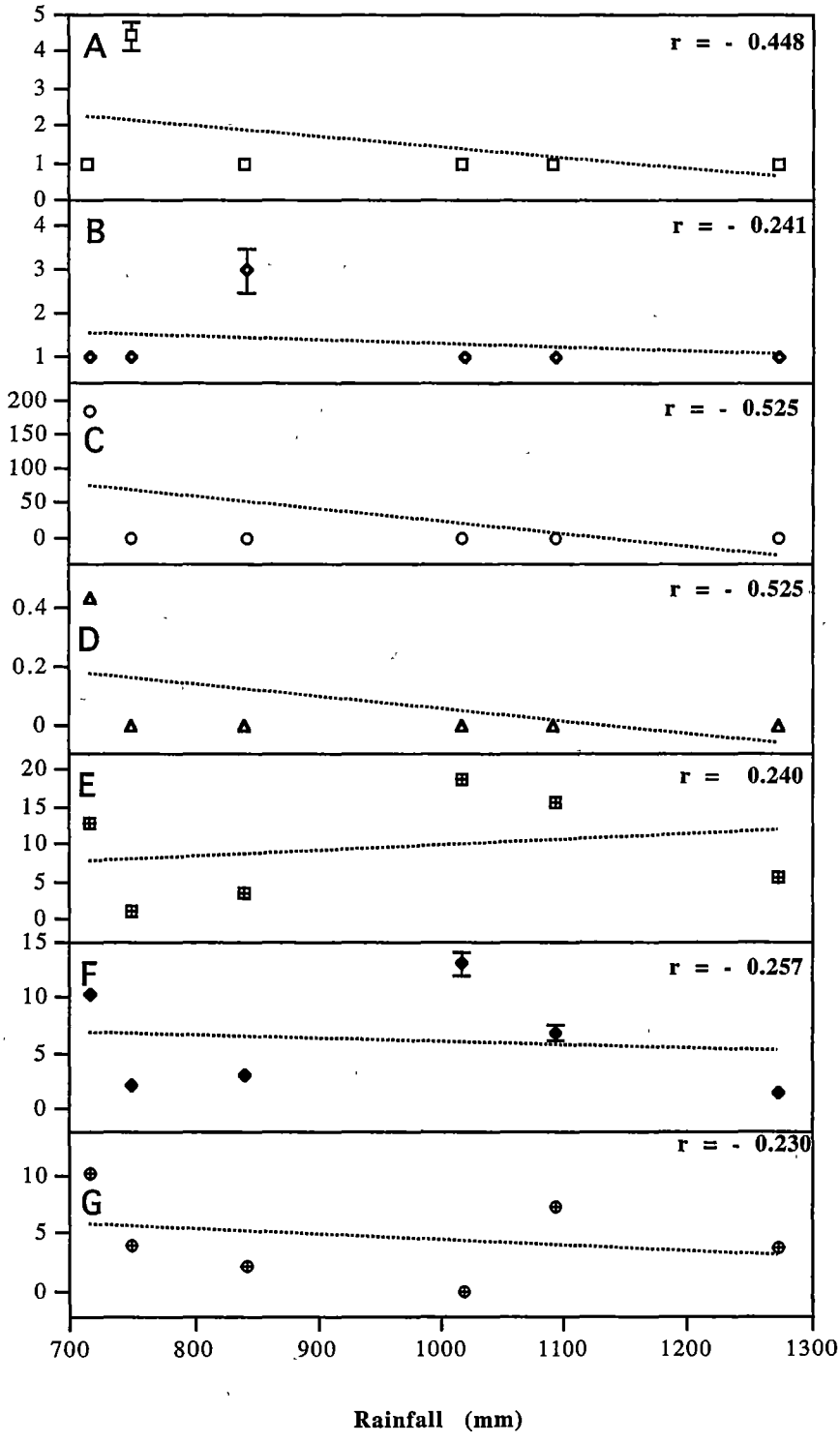

A
r = - 0.448
B
r = - 0.241
C
r = - 0.525
D
r = - 0.525
E
r = 0.240
F
r = - 0.257
G
r = - 0.230
700
800
900
1000
1100
1200
1300
Rainfall (mm)

Figure2.28. Graphs showing correlations of stomatal protection components of *Orites* species and estimated maximum of the range of habitat annual temperature.

A. Index depression *vs.* temperature. The correlation coefficient -0.019 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs.* temperature. The correlation coefficient -0.228 was found to be not significant ($p>0.1$).

C. Index hair *vs.* temperature. The correlation coefficient 0.965 was found to be highly significant ($p<0.01$).

D. Index epidermis *vs.* temperature. The correlation coefficient 0.965 was found to be highly significant ($p<0.01$).

E. Stomatal cuticle *vs.* temperature. The correlation coefficient 0.486 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle *vs* temperature The correlation coefficient 0.252 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* temperature. The correlation coefficient 0.554 was found to be not significant ($p>0.1$).

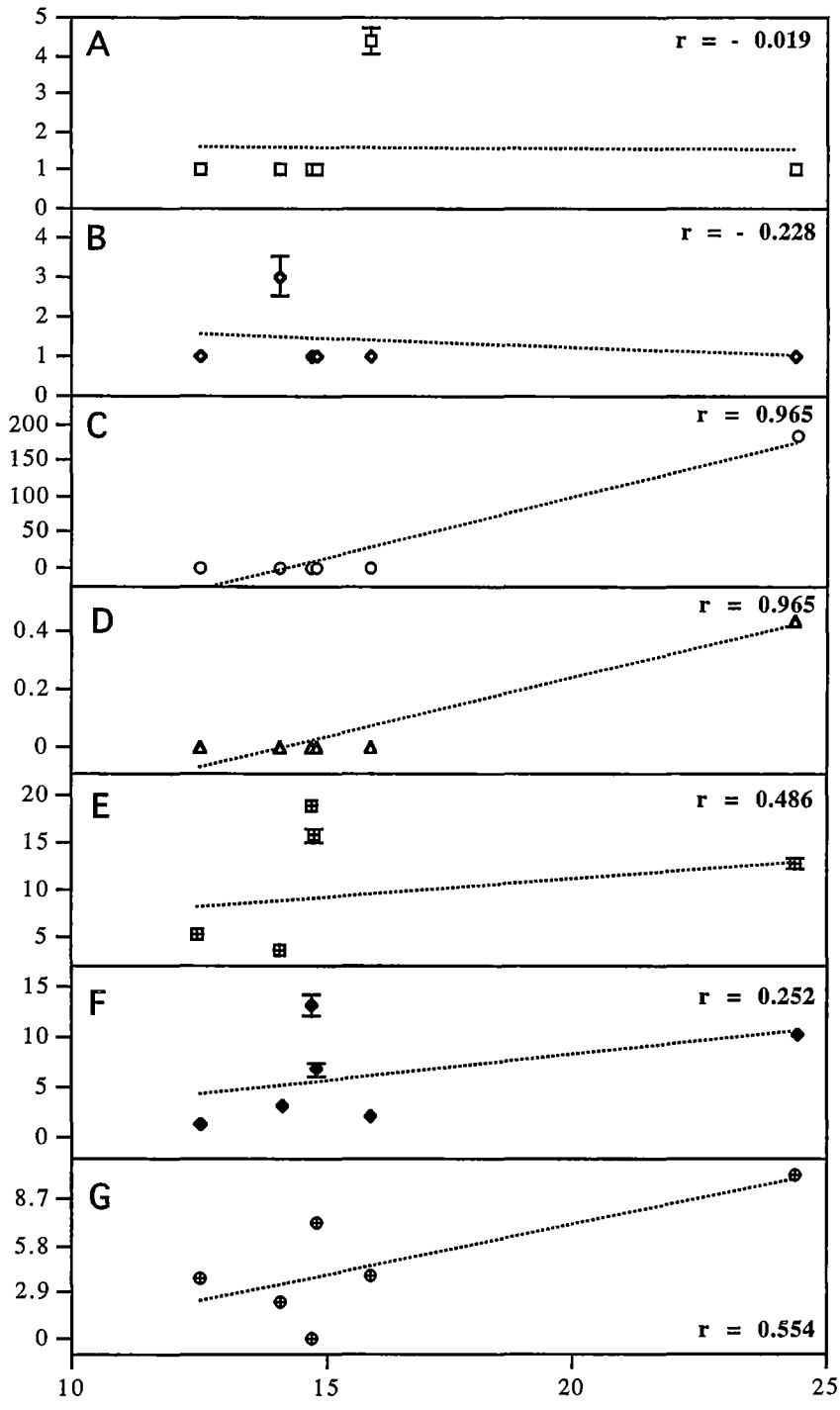

A
r = - 0.019
B
r = - 0.228
C
r = 0.965
D
r = 0.965
E
r = 0.486
F
r = 0.252
G
r = 0.554
Temperature (oC)

Figure2.29. Graphs showing correlations of stomatal protection components of *Orites* species and estimated maximum daily radiation of the habitat.

A. Index depression *vs.* radiation. The correlation coefficient -0.277 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs.* radiation. The correlation coefficient -0.277 was found to be not significant ($p>0.1$).

C. Index hair *vs.* radiation. The correlation coefficient 0.912 was found to be significant ($p<0.05$).

D. Index epidermis *vs.* radiation. The correlation coefficient 0.912 was found to be significant ($p<0.05$).

E. Stomatal cuticle *vs.* radiation. The correlation coefficient 0.142 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle *v s* radiation. The correlation coefficient 0.181 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* radiation. The correlation coefficient 0.590 was found to be not significant ($p>0.1$).

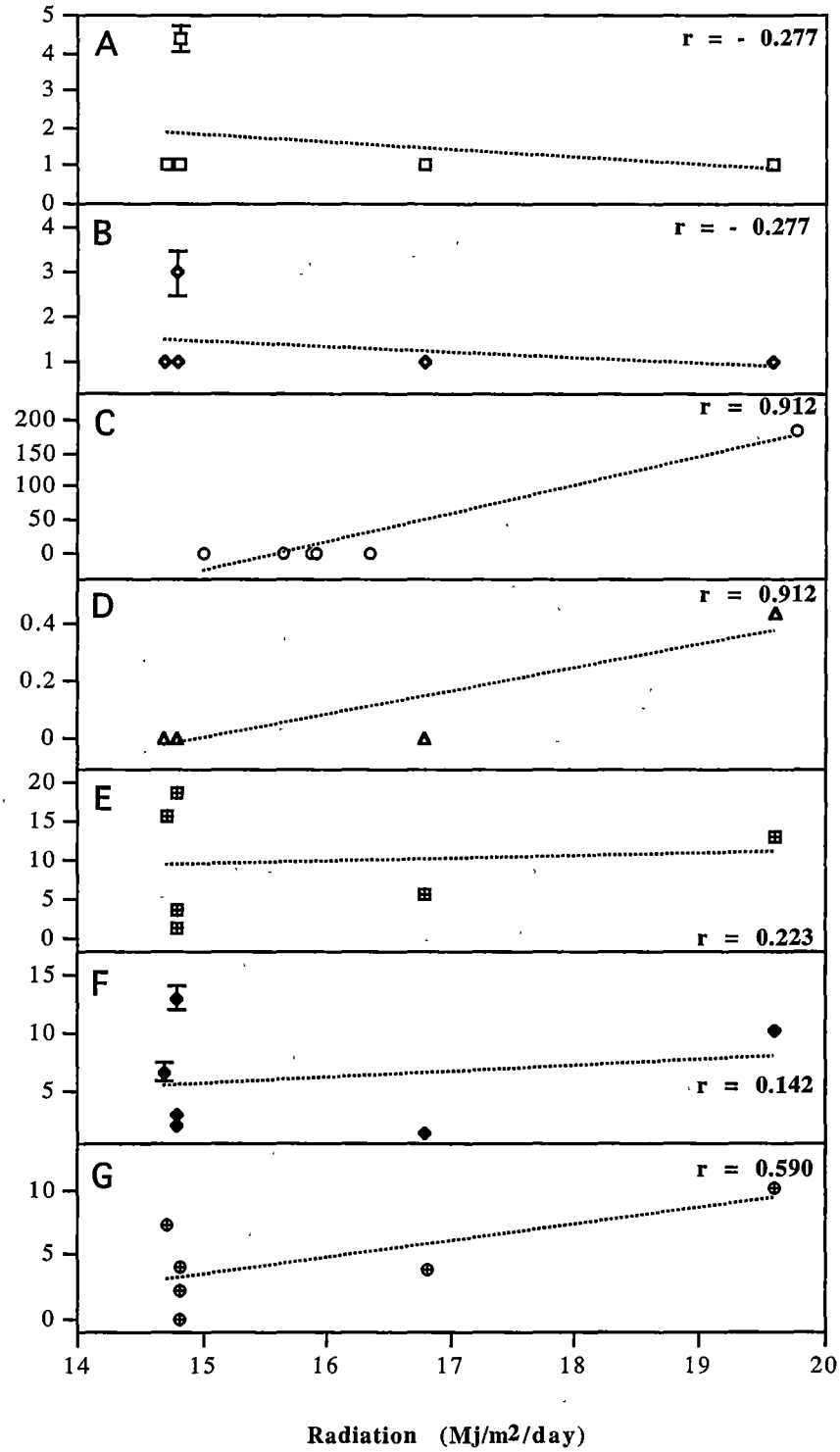

A
r = - 0.277
B
r = - 0.277
C
r = 0.912
D
r = 0.912
E
r = 0.223
F
r = 0.142
G
r = 0.590
Radiation (Mj/m2/day)

Figure2.30. Graphs showing correlations of stomatal protection components of *Orites* species and estimated maximum daily wind run of the habitat.

A. Index depression *vs.* wind run. The correlation coefficient -0.277 was found to be not significant ($p>0.1$).

B. Index recurved margin *vs.* wind run. The correlation coefficient -0.310 was found to be not significant ($p>0.1$).

C. Index hair *vs.* wind run. The correlation coefficient 0.518 was found to be not significant ($p>0.1$).

D. Index epidermis *vs.* wind run. The correlation coefficient 0.518 was found to be not significant ($p>0.1$).

E. Stomatal cuticle *vs.* wind run. The correlation coefficient -0.319 was found to be not significant ($p>0.1$).

F. Upper epidermis cuticle *vs* wind run. The correlation coefficient -0.38 was found to be not significant ($p>0.1$).

G. Lower epidermis cuticle *vs.* wind run. The correlation coefficient 0.538 was found to be not significant ($p>0.1$).

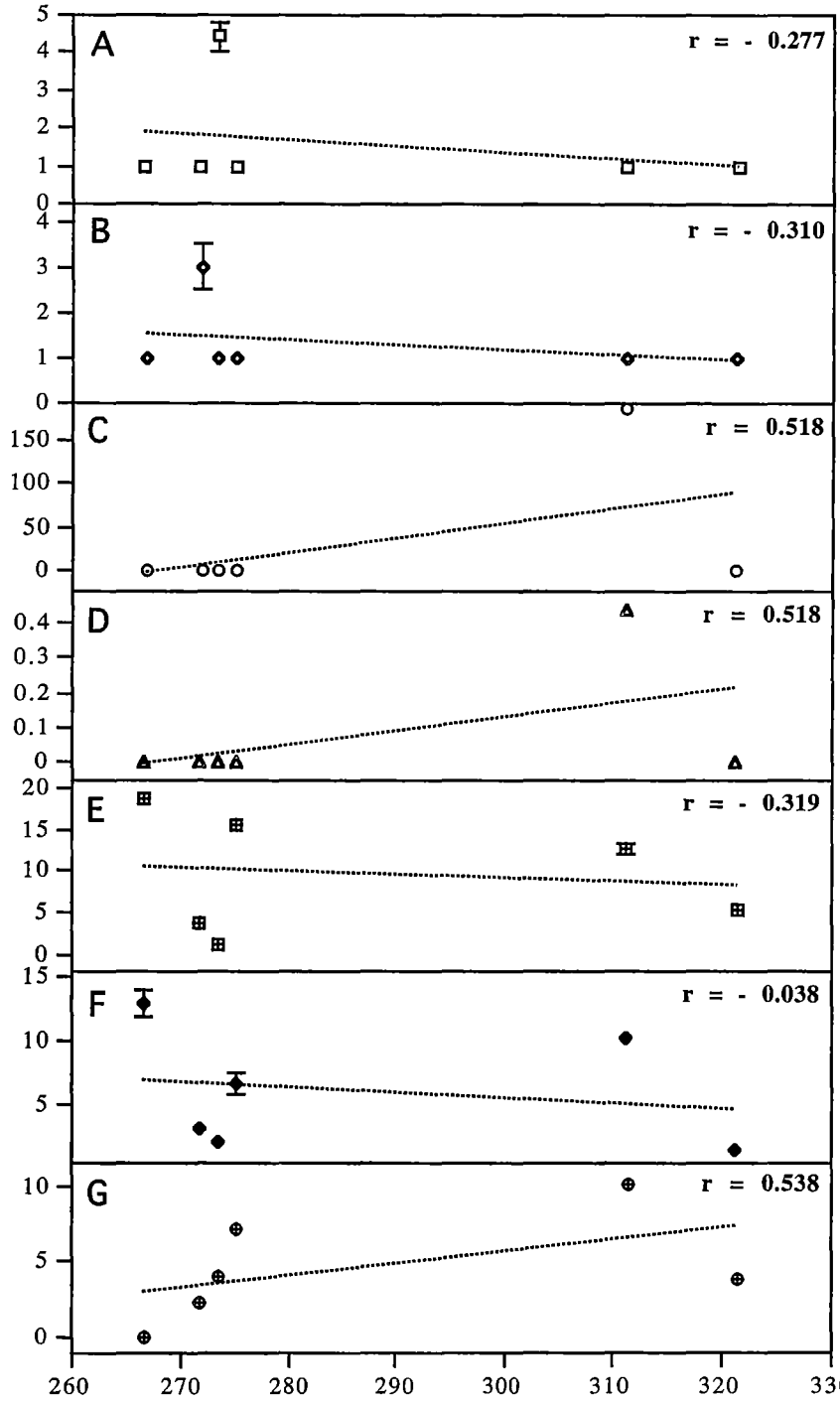

A
r = - 0.277
B
r = - 0.310
C
r = 0.518
D
r = 0.518
E
r = - 0.319
F
r = - 0.038
G
r = 0.538
260
270
280
290
300
310
320
330
Wind run (km/day)

Table 2.16. Correlation (coefficient value and probability) between character dimensions of *Orites* species and habitat climatic factors
Dim = dimensions; Rft = minimum of the range of annual rainfall for all species observed; Rf = minimum of the range of annual rainfall; T = maximum of the range of annual temperature; R = maximum of the range of daily radiation; Wr= maximum of the range of daily wind run; c = count; r = correlation coefficient; p = probability; Id = depression index; dw = depression width; dd = depression depth; Irm = index of recurved margin; Ih = hair index; hl and hd = hair length and diameter; Ie = epidermis index; de = epidermis hair density; dh = hair density; ds = stomate density; Sc, Uec and Lec = the thickness of stomatal cuticle and upper and lower epidermis cuticle.

Dim	Rf			T		R		Wr	
	c	r	p	r	p	r	p	r	p
Id	6	-0.448	>0.1	-0.019	>0.1	-0.277	>0.1	-0.277	>0.1
dw	6	-0.448	>0.1	-0.019	>0.1	-0.277	>0.1	-0.277	>0.1
dd	6	-0.448	>0.1	-0.019	>0.1	-0.277	>0.1	-0.277	>0.1
Irm*	6	-0.241	>0.1	-0.228	>0.1	-0.277	>0.1	-0.310	>0.1
Ih	6	-0.525	>0.1	0.965	<0.005	0.912	<0.05	0.518	>0.1
hl	6	-0.525	>0.1	0.965	<0.005	0.912	<0.05	0.518	>0.1
hd	6	-0.525	>0.1	0.965	<0.005	0.912	<0.05	0.518	>0.1
Ie	6	-0.525	>0.1	0.965	<0.005	0.912	<0.05	0.518	>0.1
dh	6	-0.525	>0.1	0.965	<0.005	0.912	<0.05	0.518	>0.1
ds	6	-0.597	>0.1	-0.586	>0.1	-0.482	>0.1	0.341	>0.1
Sc	6	-0.240	>0.1	0.486	>0.1	0.142	>0.1	-0.319	>0.1
Uec	6	0.257	>0.1	0.252	>0.1	0.181	>0.1	-0.038	>0.1
Lec	6	-0.230	>0.1	0.554	>0.1	0.590	>0.1	-0.538	>0.1

* the dimensions have relative values

It has already been mentioned that the presence of hairs is very important to cope with a harsh habitat in high altitude places (Table 2.16). The hair dimensions, hair length ($p<0.005$) and width ($p<0.005$), and hair density ($p<0.005$) contributed significantly to stomatal protection. These findings agree with Wuencher's (1969) findings that hairs have a role in protecting stomates and the epidermis. The glasshouse plants which were not subject to the natural environment of the species do not produce such a protective structure.

B.2. *Orites revoluta* field *vs.* glasshouse grown

Table 2.17 clearly shows that the leaves of field and glasshouse grown plants showed many differences in their characters both qualitatively and quantitatively. The most obvious difference is the absence of leaf hairs in the glasshouse plants. This fact supports the finding previously presented (Table 2.16). The thickness of the stomatal, lower and upper epidermis cuticles was highly significantly different between the plants grown in these two environments.

Table 2.17. Comparison of characters of *O. revoluta* field and glasshouse grown plants.

Characters	Field	Glasshouse	T-test	Probability
Id	0	0	-	-
Irm	2.80±0.34	3.00±0.50	0.325	0.7530
Ih($x10^2\mu m^2$)	4.01±0.47	0	7.131	0.0001
Ie	3.54±0.18	0	19.476	0.0001
Sc (μm)	1.65±0.09	2.40±0.19	-8.191	0.0001
Uec (μm)	13.1±0.29	2.16±0.17	25.465	0.0001
Lec (μm)	3.32±0.4	1.53±0.06	2.445	0.0390

C. Character Ranges

Analysis of variance for the characters measured was applied (Table 2.18). The results showed that there are very highly significant differences in the value of every cuticle thickness among the species observed. However, among the indices there was no significant variation because most of them did not possess these characters. For example, *O. diversifolia* was the only species observed showing single stomatal depressions, while *O. revoluta* was the only species observed which possessed a recurved margin. Analysis of correlation, however, found that there was no correlation ($p>0.05$) between these characters and the climatic factors.

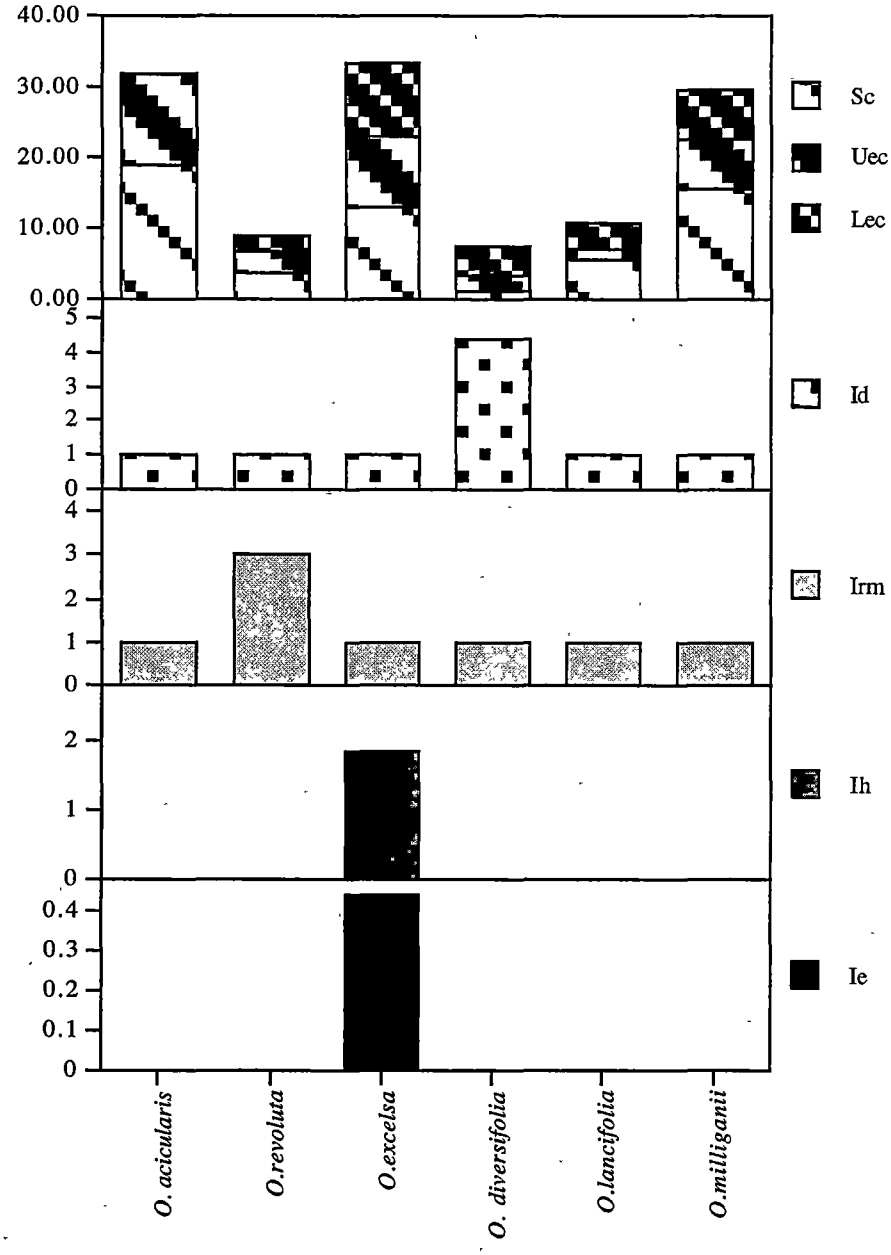


Figure 2.31. Graphs describing the variation of stomatal protection components in *Orites* species.
Id = depression index; Irm = index of recurved margin (Id and Irm - value 1 equal to 0, see page 39 - 40); Ih= hair index ($10^2\mu m^2$); Ie = epidermis index; Sc = stomatal cuticle thickness (μm); Uec = upper epidermis cuticle (μm); lec = lower epidermis cuticle (μm).

Table 2.18. Significant levels of every index calculated and cuticle thickness measured among *Orites* species observed

Index	F-test	Probability	Transformation
Depression (Id)*	-	-	-
Recurved margin (Irm)*	-	-	-
Hairs (Ih)*	-	-	-
Epidermis (Ie)*	-	-	-
Stomatal cuticle (Sc)	157.14	0.0001	log y
Upper epidermis cuticle (Uec)	426.07	0.0001	$\sqrt{y}$
Lower epidermis cuticle (Lec)	278.55	0.0001	$\sqrt{y}$

* not analysed due to no variation

It appears that the species characters assumed to increase the boundary layers are very diverse both qualitatively and quantitatively. Most species possess several of the characters measured. Figure 2.31 clearly describes the possession of characters increasing the boundary layer of every species observed.

Table 2.19a-g. Comparisons of mean values among *Orites* species observed for every index calculated. These tables describe the variety of the values of every character calculated among species observed.

a. Depression index (Id)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
O. diversifolia	5	7.838	A
O. acicularis	5	0.000	B
O. excelsa	5	0.000	B
O. lancifolia	5	0.000	B
O. milliganii	5	0.000	B
O. revoluta	5	0.000	B

b. Index of recurved margin (Irm)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
O. revoluta	5	3.354	A
O. acicularis	5	0.000	B
O. excelsa	5	0.000	B
O. lancifolia	5	0.000	B
O. milliganii	5	0.000	B
O. diversifolia	5	0.000	B

c. Hair index (Ih)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
O. excelsa	5	1.850	A
O. diversifolia	5	0.000	B
O. acicularis	5	0.000	B
O. lancifolia	5	0.000	B
O. milliganii	5	0.000	B
O. revoluta	5	0.000	B

d. Epidermis index (Ie)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
O. excelsa	5	0.436	A
O. diversifolia	5	0.000	B
O. acicularis	5	0.000	B
O. lancifolia	5	0.000	B
O. milliganii	5	0.000	B
O. revoluta	5	0.000	B

e. Stomatal cuticle (Sc)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
O. acicularis	5	12.908	A
O. excelsa	5	10.357	A
O. milliganii	5	6.576	B
O. revoluta	5	3.198	C
O. diversifolia	5	2.137	D
O. lancifolia	5	1.490	E

f. Upper epidermis cuticle (Uec)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
O. acicularis	5	18.642	A
O. milliganii	5	15.648	B
O. excelsa	5	12.715	C
O. lancifolia	5	5.475	D
O. revoluta	5	3.588	E
O. diversifolia	5	1.184	F

g. Lower epidermis cuticle (Lec)

SPECIES	n	MEAN	TUKEY (HSD) GROUPING
O. excelsa	5	10.163	A
O. milliganii	5	7.209	B
O. diversifolia	5	3.993	C
O. lancifolia	5	3.796	C
O. revoluta	5	2.298	D
O. acicularis	5	0.000	E

Leaves of *Orites* species are very interesting due to their shape variation, however, they do not have typical characters that are assumed to increase the boundary layer. *Orites diversifolia* was the only species observed having stomatal depressions. This species tended to inhabit areas with a minimum of the range of annual rainfall of 750 mm, while *O. revoluta* was the sole species observed with recurved margined leaves, and it occupied habitats with minimum of the range of annual rainfall of 842 mm.

The presence of hairs on the leaf surfaces seems to be important for this genus since this character showed strong correlations with maximum annual temperature ($r= 0.965$; $p<0.01$) and maximum daily radiation ($r=0.912$; $p<0.05$). However, it seems that presence of hairs is not a characteristic of this genus. *O. excelsa* and *O. revoluta* (field grown) are two species which possess this character, and they inhabit areas with a minimum of the range of annual rainfall of 716 mm and 842 mm, respectively.

The presence of cuticle projections on guard cells was an obvious character of this genus. Epidermis cuticles, both upper and lower, were also prominent in this genus. *O. acicularis* and *O. excelsa* and *O. milliganii* have thick stomatal cuticles. They tend to inhabit areas with 716 - 1094 mm. *O. revoluta, O. diversifolia,* and *O. lancifolia* have a low stomatal cuticle thickness and tend to inhabit areas with minimum of the range of annual rainfall ranging from 750 - 1273 mm.

Species with the thickest upper epidermis cuticles were *O. acicularis* and *O. excelsa.* They occupy areas with a minimum of the range of annual rainfall of 716 - 1018 mm. *O. milliganii* had a medium cuticle thickness, and occupies habitats with a minimum of the range of annual rainfall of 1094 mm. *O. revoluta, O. lancifolia* and *O. diversifolia* possess the thinnest upper epidermis cuticles and they inhabit areas with minimum of the range of annual rainfall of 700 - 1273 mm.

Orites excelsa possesses the thickest lower epidermis cuticle, and it tends to inhabit areas with a minimum of the range of annual rainfall of 1273 mm. *O. milliganii* has medium thickness, and occupies areas with a minimum rainfall of 1094 mm. The rest of the species have the thinnest cuticles and inhabit places with a minimum rainfall of 751 - 1273 mm.

Even though their presence in leaves is quite obvious, cuticles apparently are not important characters in respect to dryness protection. Their qualitative and quantitative variations do not seem to be a response to the habitat climatic factors.

2.4. Discussion

It was assumed that abundant trichomes (hairs), stomatal depressions, revolute leaf margins (Hill, 1998) and cuticles (Cowan, 1977) may increase the boundary layer in some Proteaceae, hence plants that possess these structures have the capability to reduce excessive transpiration. It was found that *Banksia* and *Grevillea* leaves have typical characters increasing the boundary layer, however *Orites* does not. This present research tries to find further evidence for this assumption, by examining character variation within and among the genera observed and correlating the characters scored for *Banksia, Grevillea* and *Orites* with some habitat climatic factors. The result showed that there is a large variation in these character values, however when they were correlated with their habitat climatic factors most of the correlations revealed no significant correlation. Due to the narrow ranges of most of the habitat climatic factors of the species representatives, the trend of the correlation was used as the main consideration in predicting the tendency of an association. The results are elaborated in detail in the following sections.

1. Stomatal depression

Stomatal depressions were found to be very common in *Banksia* (Figure 2.13) and *Grevillea* (Figure 2.20), and only occasional in *Orites* (Figure 2.26). However there were differences in the number of stomates protected within each depression. In *Grevillea* and *Orites* a depression protects stomates individually. Usually the depression is constructed by epidermal cells and epidermis cuticle projections (*e.g.* Figure 2.20a-G1, O4). On the other hand, in *Banksia,* a depression protects more than one stomate, and it is built as an interveinal depression (*e.g.* Figure 2.13b-B4). Seemingly, stomatal depressions in *Banksia* are more protective than those in *Grevillea,* but no physiological experimentation has been done in this area.

Stomatal depressions of *Banksia* can be classified into 3 categories on the basis of their shapes, namely U (Figure 2.13h-B23), V (Figure 2.13a-B1) and balloon-like shapes (Hill, 1994)(Figure 3.1c). Those of *Grevillea* and *Orites* cannot be classified in this way as the shapes are almost uniform. The U shapes have values for the ratio of median to surface areas of around one, V shapes have ratios of less than one and the balloon-like shapes have ratios of more than one. The U and V-shaped depressions are commonly found, but the balloon-like-shaped ones are only found in species that

inhabit extreme conditions. It was assumed that the smaller the surface areas and the deeper the depressions the better the protection for the stomates.

More than 50% (Table 2.20) of the species observed in *Banksia* and *Grevillea* possessed stomatal depressions. It was not considered as a common character in *Orites*, because all of the species observed inhabit areas with a minimum range of annual rainfall varying from 716 - 1273 mm. *Orites diversifolia* was the only species which showed this structure, and it inhabits areas with a minimum range of annual rainfall of 750 mm. Habitats of the *Orites* species observed were potentially wet, because most of them occupy alpine areas (Table 2.15), hence they do not have to cope with lack of water regularly. In winter, however, they might experience other stress conditions, which force them to adapt by possessing the given structure. Furthermore, there is a possibility that the given structure plays a role as a protector from a severe climatic factor other than drought in these areas.

Despite its common presence in *Banksia* and *Grevillea*, there is no significant correlation between depression index and habitat annual rainfall in any genus observed. The statistical analysis did not indicate any significant correlation and this is possibly due to the narrow range of most of the habitat climatic factors of the species observed. There is, however, a tendency for the given character to have a negative correlation with the minimum of the range of annual rainfall. This means that it shows the response hypothesized (Hill, 1994) meaning that species growing in dryer habitats tend to have deeper stomatal depressions. Nevertheless, it was obvious that the variation in the minimum of the range of annual rainfall of the habitat of species observed has not stimulated different responses, it thus could not indicate a significant correlation. A similar adaptation has already been observed in some more recent fossil deposits, e.g. leaves of the Pleistocene *Banksia kingii* (Jordan and Hill, 1991). These results are in accordance with Blackburn's (1985) finding in *Banksieaephyllum* spp. from the Latrobe Valley coal, which showed little or no prominent stomatal protection for species associated with rainforest.

Depression index variation in *Grevillea* and *Orites* did not reveal any association with the maximum annual temperature of the habitats ($p>0.1$), although they displayed positive correlation trends. *Banksia* significantly correlated with the maximum annual temperatures ($p<0.05$). Structures which significantly support the performance of stomatal depressions are the areas of surface and median plates, and depression depth ($p<0.05$). Seemingly, the more hidden stomates are the less exposed to high temperatures they are, and as a consequence they do not transpire excessively. This

result agrees with Wood's (1923) results for the transpiration of *Geijera parviflora* growing in the arid zone of South Australia. The ridges in leaves of this species have a role in reducing transpiration, even though temperature, evaporating power and light intensity are high.

Table2.20. Species groupings based on depression index values (Id) in accordance with Tukey test results for every genus observed

Genus	Classes of Id	Species
Banksia	No depression	*B. conferta* var. *conferta, B. laevigata* ssp.*laevigata, B. brownii, B. occidentalis, B. nutans* var. *cernuella, B. ericifolia var. ericifolia, B. integrifolia* var. *integrifolia, B. seminuda, B. dryandroides, B. spinulosa* var. *spinulosa, B. verticillata*
	0.80 - 1.60	*B. media, B. aemula, B. ashbyi, B. sceptrum, B. baxteri, B. serrata, B. attenuata, B. baueri, B. praemorsa, B. lemanniana*
	1.61 - 2.40	*B. robur*
	>2.41	*B. caleyi*
Grevillea	No depression	*G. aspera, G. lavandulacea, G. spinosissima, G. lissopleura, G. beadleana, G. iaspicula, G. caleyi, G. willisii.*
	<10.68	*G. johnsonii, G. aquifolium, G. manglesii* ssp. *dissectifolia, G. bipinnatifida, G. sericea, G. phanerophlebia, G. nudiflora.*
	10.69 - 17.98	None
	>17.99	*G. pilosa* ssp. *pilosa*
Orites	0	*O. acicularis, O. revoluta, O. excelsa, O. lancifolia, O. milliganii*
	7.84	*O. diversifolia*

The depression index for every genus observed did not display any significant correlation with the wind run. The trends, however, showed a negative correlation. This is an interesting finding, since logically positioning of stomates on the leaf surface exposes them more to the breeze. Thus, the deeper the stomatal position, the less exposed the stomates are. This is in agreement with Bange's (1953, in Noggle and

Fritz (1976)) finding that in moving air the water vapour is blown away relatively quickly. On the other hand, in still air the water vapour collects above stomates, in a boundary layer.

2. Recurved margins

Recurved margins are predominant in *Grevillea* (83.3%). Some *Banksia* species observed (26.8%) and *Orites revoluta* also possess this structure, however they are not as prominent as those in *Grevillea*. Recurved margins behave like stomatal depressions, yet their length extends to the whole length of a leaf. In cross sections most of them seem to have the balloon-like shape with very narrow surface areas. Such recurvations seem to indicate strong stomatal protection, however due to the variation of most habitat climatic factors which has not stimulated variation in the character observed, the statistical analysis did not show any significant correlation. The correlation of the index of recurved margin with temperature in *Banksia* and *Orites* displayed negative trends, but in *Grevillea* it showed the opposite trend. This phenomenon of extant *Banksia* was in accordance with that of *Banksia strahanensis* from the West Coast of Tasmania. Leaves of species with a very long and narrow revolute margin indicate well-developed xeromorphic adaptations (Jordan and Hill, 1991).

It seems logical that recurved margins play a role in protecting the stomates. However, the results of this study demonstrated that variation in the minimum of the range of annual rainfall of the habitat did not contribute to variation in the values of recurved margin index, as indicated by the low level of correlation between them (Tables 2.7, 2.12, 2.16). Therefore, it is likely that in the *Grevillea* species observed, recurved margins are not tools for protecting stomates from habitat dryness. Yet, by considering their prominent presence in this genus, it is very possible that this character has other roles to keep this taxon existing in its habitat.

Another interesting finding in *Grevillea* showed that the correlation coefficient between recurved margin and the maximum of the range of annual temperature (0.048) was found to be not significant ($p>0.1$). This means that the variation in the recurved margins is not a reliable indicator of the maximum of the range of annual temperature differences. On the contrary, in *Banksia* and *Orites*, negative trends of the correlation were observed. This means that in both genera the differences of the recurved margin values do not associate well with temperature variations.

Seemingly, wind run is not a significant habitat factor, and its variation should not be accounted for in relation to plant adaptation. In none of the genera observed did the index of recurved margin show a strong correlation with the maximum of the range of daily wind run of its habitat. Moreover, in *Banksia*, the index of recurved margin revealed a negative correlation with wind run, which is contradictory to Bange's finding (1953), as previously mentioned.

Table 2.21. Species groupings based on the index of recurved margin values (Irm) in accordance with Tukey test results for every genus observed

Genus	Classes of Irm	Species
Banksia	No recurvation	*B. baxteri, B. attenuata, B. brownii, B. aemula, B. ashbyi, B. integrifolia* var.*integrifolia, B. baueri, B. lemanniana, B. media, B. caleyi, B. conferta* var. *conferta, B. praemorsa, B. robur, B. sceptrum, B. seminuda, B. serrata, B. spinulosa* var. *spinulosa, B. verticillata*
	1.01-2.00	*B. nutans* var. *cernuella, B. dryandroides, B. laevigata* ssp. *laevigata, B. occidentalis, B. baxteri*
	>2.00	*B. ericifolia* var. *ericifolia*
Grevillea	No recurvation	*G. aquifolium, G. pilosa* ssp. *pilosa, G. phanerophlebia.*
	<4.85	*G. manglesii* ssp. *dissectifolia, G. sericea, G. bipinnatifida, G. iaspicula, G. nudiflora, G. johnsonii, G. lavandulacea, G. caleyi, G. aspera, G. willisii, G. beadleana,*
	4.86 - 8.61	*G. lissopleura*
	>8.62	*G. spinosissima*
Orites	0	*O. acicularis, O. excelsa, O. diversifolia, O. lancifolia, O. milliganii*
	3.35	*O. revoluta*

3. Hairs

The role of hairs as radiation protectors (Ehleringer and Björkman, 1978; Gausman and Cardenas, 1969) has been widely accepted, apart from their other functions as

water absorbers (Lyshede, 1976), water repellents (Brewer and Smith, 1997) and water protectors (Duddington, 1969). This structure is prominent in *Banksia* and *Grevillea*, but not in *Orites*. Among species observed, all *Banksia* species possess hairs, 88.8% in *Grevillea* and 16.6% in *Orites* (Table 2.22).

Hairs are interesting structures, since their presence in plants does not always take the same role. This could lead to some confusion. For this reason integrated research on anatomy-physiology and ecology is necessary in order to provide clear evidence of the role of hairs in certain taxa.

An interesting result, which is in contrast to the generally hypothesized role of hairs, was found in *Grevillea*. The hairs, which are assumed to have a function as stomatal protectors from dryness, showed a positive trend when they were correlated with the minimum of the range of annual rainfall of the habitat. This means that increasing habitat wetness is followed by increasing hair densities and dimensions (length and diameter).

It is possible that hairs still play a similar role, that is as stomatal protectors, but in this case they protect them from excessive wetting, and hence the stomates are not flooded. This argument is supported by the results of Brewer and Smith (1997), showing that foliar hairs have a large role in reducing wetness and retaining droplets on the leaf surface, particularly on the stomate-rich surface.

In *Banksia*, the correlation between hair index and the minimum of the range of annual rainfall showed a negative trend, whereas the correlation with the maximum of the range of daily wind run, showed a positive trend. This evidence may give more information about the roles of hairs in *Banksia*. It seems that the variation among species observed is not a response to the differences in habitat dryness. However, their presence clearly has a protective role, since it has been reported that the average reflectance of leaves increased (absorptance decrease) as the aridity of the habitat increased. Based on these findings, it can be concluded that xeromorphic leaves tend to have pubescent surfaces. Such surfaces reflect solar radiation, thus reducing leaf temperature, and the lower temperature reduces leaf transpiration.

4. Epidermis

The epidermis index is defined as the ratio of the number of hairs to stomate number. This idea has direct correlation with the role of hairs as protection against water loss.

The rationale is, if the epidermis index value is larger, the stomates are more protected, without regard to the stomate size. In *Banksia*, 100% of species observed possess relatively high epidermis index values, with two species, *B. spinulosa* var. *spinulosa* and *B. nutans* var. *cernuella* having the largest ones (Table 2.23). Compared to those in *Grevillea*, in *Banksia* epidermis index values tend to be larger. It is possibly because *Banksia* usually has denser hairs but less stomates per μm^2 than *Grevillea*. Two species of *Grevillea* do not possess any hairs, and hence they have no value of epidermis index.

Table 2.22. Species groupings based on hair index values (Ih, μm^2) in accordance with Tukey test results for every genus observed

Genus	Classes of Ih	Species
Banksia	< 5.75	*B. praemorsa, B. ashbyi, B. integrifolia* var. *integrifolia, B. conferta* var. *conferta, B. caleyi, B. media, B. baxteri, B. serrata, B. lemanniana, B. baueri, B. robur, B. aemula. B. sceptrum,*
	5.76 - 11.50	*B. attenuata, B. laevigata* var. *laevigata, B. seminuda, B. dryandroides, B. ericifolia* var. *ericifolia, B. verticillata*
	>11.51	*B. occidentalis, B. spinulosa* var. *spinulosa, B. brownii, B. nutans* var. *cernuella.*
Grevillea	No hair <9.39	*G. iaspicula, G. manglesii* ssp. *dissectifolia. G. aquifolium, G. sericea, G. spinosissima, G. phanerophlebia, G. pilosa* ssp. *pilosa. G. lissopleura, G. nudiflora, G. johnsonii G. bipinnatifida,*
	9.40 - 18.78	*G. aspera, G. .lavandulacea, G. beadleana, G. willisii.*
	>18.79	*G. caleyi.*
Orites	0	*O. acicularis, O. revoluta, O. diversifolia, O. lancifolia, O. milliganii*
	185	*O. excelsa*

Within *Banksia*, it was found that there was no significant correlation between the minimum of the range of annual rainfall and either depression index, frequency of depression hairs, frequency of epidermis hairs, or frequency of stomates. Similar

findings were obtained in *Grevillea,* whereas *Orites* showed different results. Both *Banksia* and *Grevillea* possess abundant hairs (Figures 2.13, 2.20) but the hair dimensions are very diverse, in which the differences do not seem to respond to the variation of the habitat rainfalls. It is possibly because the range of the rainfall is quite narrow so that it does not stimulate structural modifications. This result seems to create doubt about the roles of hairs in these genera, however some findings showed positive results for the role of hairs in stomatal protection.

Similar findings for *Banksia* and *Grevillea* are shown in the correlation between epidermis index values and either the maximum of the range of annual temperature, maximum of the range of daily radiation or maximum of the range of daily wind run. Presumably, these habitat climatic factors behave like the minimum of the range of annual rainfall. However, some interesting findings in *Banksia* occurred in response to the maximum of the range of annual temperature and maximum of the range of daily radiation.

Positive trends in correlations were found between both frequency of epidermis hairs (0.451) and stomates (0.541), and the maximum of the range of annual temperature. These phenomena indicate that epidermis hairs could have a large role in protecting stomates from increasing temperature which can also be the result of increasing irradiation as indicated by positive trends of correlation between both frequency of epidermis hairs (0.360) and stomates (0.628), and the maximum of the range of daily radiation. These facts suggest that possibly epidermis hairs play a role in reflecting unnecessary light and reducing temperature as well. Presumably, the presence of thicker cuticle on the epidermis hairs could reflect the incoming radiation and thus reduce the temperature. This argument agrees with Lyshede's (1976) findings on the structure and function of hairs in *Spartocytisus filipes.* Since the hairs have shiny surfaces which reflect sunrays, they protect the chloroplasts from overheating.

In *Orites,* strong and significant correlations between epidermis index and both maximum of the range of annual temperature (0.965; $p<0.005$) and maximum of the range of daily radiation (0.912; $p<0.05$) were observed. As was previously discussed, hairs have significant roles in protecting the stomates from high radiation and high temperature. Moreover, there is a positive trend in the correlation between epidermis index and maximum of the range of daily wind run. This suggests that hairs have another role to inhibit the breeze that sweeps the water vapours away from the stomates (Duddington, 1969).

Table 2.23. Species groupings based on epidermis index values (Ie) in accordance with Tukey test results for every genus observed

Genus	Classes of Ie	Species
Banksia	< 1.20	*B. serrata, B. conferta* var. *conferta, B. ericifolia* var.*ericifolia, B. robur B. integrifolia* var.*integrifolia, B. baxteri, B. praemorsa, B. baueri. B. media, B. attenuata, B. lemanniana, B. caleyi, B. sceptrum, B. ashbyi.*
	1.21 - 2.40	*B. dryandroides, B. aemula, B. verticillata, B. seminuda, B. occidentalis, B. brownii, B. laevigata* ssp laevigata, *B. brownii B. verticillata.B. seminuda, B. occidentalis,*
	>2.41	*B. spinulosa* var. *spinulosa, B. nutans* var. *cernuella.*
Grevillea	No hair	*G. iaspicula, G. manglesii* ssp. *dissectifolia.*
	<0.55	*G. spinosissima, G. phanerophlebia, G. lissopleura, G. bippinatifida.*
	0.56- 1.11	*G. aquifolium,G. sericea, G. pilosa* ssp. *pilosa, G. nudiflora, G. beadleana, G. caleyi, G. willisii.G. aspera. G. johnsonii.*
	>1.12	*G. lavandulacea.*
Orites	0	*O. acicularis, O. revoluta, O. diversifolia, O. lancifolia, O. milliganii*
	0.44	*O. excelsa*

5. Stomatal Cuticles

It has already been assumed that cuticles reduce water loss. In leaves cuticles can be classified morphologically into three groups on the basis of their position: stomatal cuticles, which layer the surface of guard and subsidiary cells, the upper and lower epidermis cuticle. All of them are always present in every leaf observed, however, the thicknesses are different to each other and between leaves. In *Banksia,* the leaves tend to have thinner cuticles than those of *Grevillea* (Table 2.24). It might be because *Banksia* leaves have prominent stomatal depressions and abundant curly hairs (Figures 2.13). *Orites* is an interesting genus as it does not have a typical leaf

structure, and some species possess thick cuticles whilst others do not. The variation in cuticle thickness is assumed to be associated with differences in habitat climatic factors. Some evidence is proposed to support this argument.

There is no significant correlation between stomatal cuticle thickness and any habitat climatic factors observed. In *Banksia*, the correlation between stomatal cuticle and the minimum of the range of annual rainfall showed negative trends. This result is in agreement with Cowan's (1977) finding that 77% of total evaporation occurs through guard and subsidiary cells. Therefore, a thicker stomatal cuticle might help plants adapt to dryness by reducing evaporation. However, an opposite argument was proposed by Parker (1968), that cuticle and wax are prominent structures in xeromorphic leaves, but their presence does not ensure the cessation of transpiration (Kozlowski, 1968). This idea is based on Oppenheimer's (1960) finding that when the cuticle dries out, there is a constriction in the submicroscopic channels, which pass through the cuticle and this allows water to be lost. Correlations of stomatal cuticle with other habitat climatic factors, namely the maximum of the range of annual temperature, of daily wind run and of daily radiation also showed negative trends. However, the correlations are relatively small, suggesting there is no direct association between them.

In *Grevillea*, stomatal cuticle may play a greater role than in *Banksia* which is indicated by a higher thickness (Table 2.24) and a significant correlation between stomatal cuticle with the minimum of the range of annual rainfall (Table 2.12). However, *Orites* did not exhibit similar responses. It is possible that this genus does not face any problem with conserving water, due to most of the species inhabiting areas with high annual rainfall.

6. Upper Epidermis Cuticle

The upper epidermis cuticle is not a conspicuous structure in *Banksia* (Figures 2.13), and most of the species observed (65.2%) possess thin upper epidermis cuticle (Table 2.25). On the other hand, in *Orites* and *Grevillea* this structure is prominent (Figures 2.20, 2.26) with the thickness of the upper epidermis cuticle three to four times that of *Banksia* (Table 2.25). In *Banksia*, the upper epidermis cuticle did not associate well with any habitat climatic factor (Table 2.7). The presence of this structure seems to be not as important as depressions or hairs, in respect to stomatal protection. Whilst in *Orites*, even though the presence of this cuticle is quite prominent no significant correlation between upper epidermis cuticle thickness and any habitat

climatic factor was observed. This evidence indicates that the variation of the thickness of upper epidermis cuticle might not be a response to any habitat climatic factors determined.

Table 2.24. Species groupings based on the thickness of stomatal cuticle (Sc, μm) in accordance withTukey test results for every genus observed

Genus	Classes of Sc	Species
Banksia	<3.00	*B. sceptrum, B. ericifolia* var. *ericifolia,B. nutans* var. *cernuella, B. brownii,B. attenuata, B. baxteri, B. occidentalis,B. baueri, B. aemula, B. seminuda, B. spinulosa* var. *spinulosa, B. laevigata* ssp. *laevigata, B. media, B. lemanniana, B. conferta* var. *conferta*
	3.01 - 4.81	*B. ashbyi, B. robur, B. serrata, B, integrifolia* var. *integrifolia, B. dryandroides, B. caleyi.*
	>4.82	*B. praemorsa, B. verticillata.*
Grevillea	< 9.50	*G. manglesii* ssp. *dissectifolia, G. beadleana, G. spinosissima, G. iaspicula, G. nudiflora, G. sericea, G. johnsonii, G. caleyi.*
	9.51 - 16.20	*G. aquifolium, G. bipinnatifida, G. pilosa* ssp. *pilosa, G. phanerophlebia, G. willisii, G. lavandulacea.*
	>16.21	*G. lissopleura, G. aspera.*
Orites	< 6.25	*O. revoluta, O. diversifolia, O. lancifolia*
	6.26 - 12.50	None
	>12.51	*O. acicularis, O. excelsa, O. milliganii.*

Nevertheless, some possibility for the existence of a role for the upper epidermis cuticle as a radiation reflector or water saver should be noted, since in the three genera observed the upper epidermis cuticles showed a weak positive correlation with the maximum of the range of daily radiation (Tables 2.7, 2.12, and 2.16). This argument is proposed on the basis of most the findings in this area, which provided evidence that cuticle has a role in saving water, either by direct or indirect actions. A study on plants growing in excessive light and inadequate light habitats reported that the function of cuticle is not only to protect the plants from excessive transpiration, but also to reflect strong radiation on the leaf surface (Martin and Juniper, 1970). In addition, another observation on 25 species of *Eriope* conducted by Rudall (1979)

reported that the *Eriope* species, which do not have dense hairs, often protected their leaves with well-developed cuticles.

Table 2.25. Species groupings based on the thickness of the upper epidermis cuticle (Uec, μm) in accordance with Tukey test results for every genus observed

Genus	Classes of Uec	Species
Banksia	<0.53	*B. dryandroides, B. brownii, B. aemula, B. spinulosa* var. *spinulosa, B. ericifolia* var. *ericifolia.*
	0.54 - 0.69	*B. nutans* var. *cernuella, B. ashbyi, B. attenuata, B. serrata, B. media.*
	>0.70	*B. verticillata, B. baueri, B. integrifolia* var. *integrifolia, B. occidentalis, B. lemanniana, B. laevigata* ssp. *laevigata, B. caleyi, B. robur,B. praemorsa, B. sceptrum, B. conferta* var. *conferta, B. seminuda, B. baxteri*
Grevillea	< 5.50	*G. caleyi, G. iaspicula, G. johnsonii. G. beadleana, G. aquifolium, G. lissopleura, G. willisii, G. aspera, G. sericea,G. nudiflora, G. lavandulacea, G. spinosissima. G. manglesii* ssp *dissectifolia.*
	5.51 - 10.66	None
	10.67- 15.82	*G. bipinnatifida, G. phanerophlebia.*
	>15.83	*G. pilosa* ssp. *pilosa,*
Orites	< 4.50	*O. lancifolia, O. diversifolia, O. revoluta*
	4.51 - 9.00	*O. milliganii*
	> 9.01	*O. excelsa, O. acicularis.*

The upper epidermis cuticle thickness in *Grevillea* supports the above findings. Correlation between the thickness of the upper epidermis cuticle with the minimum of the range of annual rainfall revealed negative trends. This finding indicates that the fluctuation in the minimum of the range of annual rainfall might contribute to the variation in the thickness of the upper epidermis cuticle. The role of this structure is likely to replace that of other structures, hence the maximum stomatal protection is achieved.

7. Lower Epidermis Cuticles

The lower epidermis cuticle seems to have a more important role in stomatal protection. This is possibly because the cuticle position is adjacent to the stomates which are usually positioned on the abaxial surface, hence the protection is more effective. Similar to the previous findings, *Banksia* does not have any prominent lower epidermis cuticle, in fact all of the species observed have a cuticle that is less than 1μm thick. In *Grevillea* and *Orites* the cuticle thickness is greater than that of *Banksia* (Table 2.26). It is logical that *Banksia* does not require this structure, since on the lower surface it is already equipped with dense and curly hairs. However, despite the presence of hairs, the correlation between the thickness of the lower epidermis cuticle and the minimum of the range of annual rainfall demonstrated a negative trend.

In *Grevillea* and *Orites*, the lower epidermis cuticle is a prominent structure. Presumably, this structure is important in respect to stomatal protection. In *Grevillea*, significant correlations between the thickness of the lower epidermis cuticle either with the minimum of the range of annual rainfall (-0.688; $p<0.05$) or with maximum of the range of daily radiation (0.806; $p<0.05$) were observed. These findings indicated that the variation in thickness of the lower epidermis cuticle associates well with the fluctuation in habitat daily radiation and temperature.

In *Orites*, no significant correlation was observed, but the trends of correlation between the thickness of the lower epidermis cuticle and either the maximum of the range of annual temperature (0.544; $p>0.1$), or the maximum of the range of daily radiation (0.590; $p>0.1$) were observed. This means that the variation in the annual temperature and daily radiation do not contribute to the differences in the thickness of the lower epidermis cuticle. These findings suggest a good association between *Orites* and the habitat climatic factors. Because most of the *Orites* species live in alpine areas, this structure also protects stomates from excessive light and heat.

Table 2.26. Species groupings based on the thickness of the lower epidermis cuticle (Lec, μm) in accordance with Tukey test results for every genus observed

Genus	Classes of Lec	Species
Banksia	< 0.80	*B. attenuata, B. lemanniana, B.brownii, B. dryandroides, B. nutans* var. *cernuella, B. ericifolia* var. *ericifolia,*
	0.81 - 1.20	*B. praemorsa, B. ashbyi, B. integrifolia, B. sceptrum, B. conferta* var. *conferta, B. caleyi, B. media, B. laevigata* ssp. *laevigata, B. baxteri, B. serrata, B. seminuda, B. baueri, B. robur, B. verticillata, B. spinulosa* var. *spinulosa, B. aemula.*
	> 1.21	*B. seminuda*
Grevillea	<0.40	*G. willisii, G. johnsonii, G. sericea, G. caleyi, G. lavandulacea, G. beadleana, G. lissopleura.*
	0.41 - 0.80	*G. manglesii* ssp. *dissectifolia, G. bipinnatifida, G. spinosissima, G. iaspicula, G. pilosa* ssp. *pilosa, G. phanerophlebia, G. aspera.*
	>0.81	*G. aquifolium, G. nudiflora*
Orites	< 3.40	*O. acicularis, O. revoluta*
	3.41 - 6.80	*O. diversifolia, O. lancifolia*
	> 6.81	*O. excelsa, O. milliganii*

2.4. Conclusion

The results of this research show the structural diversity of the stomatal protection mechanisms in *Banksia, Grevillea* and *Orites*. Furthermore, analysis of correlations was conducted in order to obtain detailed information about the possibility of direct or indirect association between the structures assumed to increase the boundary layers and variation in cuticle thickness with habitat climatic factors.

The strategies to increase boundary layers by morphological adaptations, vary among the genera observed. Every genus has typical characters that might increase the boundary layer; in *Banksia,* stomatal depressions and hairs are prominent; in *Grevillea,* recurved margins and hairs are conspicuous; yet, in *Orites,* no typical characters were observed, with the possible exception of hairs and cuticles. Within each genus, there was not much character diversity, but there was size diversity.

Links between characters that increase the boundary layer and habitat climatic factors could not be absolutely represented, due to low sample numbers. However, the tendency of correlations is considered to be representative.

In *Banksia* stomatal depression seems to have an important role to protect the stomates from the effect of habitat climatic factors, particularly from temperature and radiation effects. The presence of recurved margins and hairs are not as important as depressions as indicated by low values of correlation coefficients. It is also likely that the density of hairs and stomates makes little contribution to the stomatal protection. There is a general weak correlation between cuticle thickness and any habitat climatic factor. However, it seems that stomatal cuticle thickness could vary according to the minimum of the range of annual rainfall.

Unlike *Banksia* species, most *Grevillea* species are characterised by cuticles playing a significant role in stomatal protection. The thickness of the stomatal and lower epidermis cuticle showed greater links to habitat climatic factors than the thickness of the upper epidermis cuticle. Also, in this genus, hairs behave differently, *i.e.* the higher the minimum of the range of annual rainfall, the greater the hair dimension. Other characters, namely depressions, revolute margins, hair and stomatal densities do not seem to contribute to the protection of stomates.

Compared to *Banksia* and *Grevillea, Orites* has unique leaf characters. There is a general strong association with habitat climatic factors for hair dimensions and densities, and also the stomate densities. The hair characters make significant contributions to protecting stomates from temperature and radiation effects. Yet, several other characters, namely depression, stomatal and lower epidermis cuticles only make a small contribution to stomatal protection. Seemingly, only recurved margins give no contribution to protecting stomates.

To sum up, it is obvious that the main strategies applied to protect stomates differ among Proteaceae members, as indicated by both morphological observations and analysis of correlations. Leaves of *Banksia* species have depressions which protect stomates from the heat. *Grevillea* species have leaves with thick cuticles, which protect stomates from excessive transpiration. Whilst in *Orites*, leaf hairs protect stomates from heat and incoming light. In general, presumably, all of the structural strategies applied in these genera, directly and indirectly contribute to reduce excessive transpiration.

Chapter 3

Transition of the Stomatal Protection Mechanisms from Juvenile to Adult Leaves in Some *Banksia* and *Grevillea* species

3.1. Introduction

Ontogeny of woody plants involves a sequence of physiological and morphological changes during plant development from embryo through juvenile and mature stages. Some plants exhibit marked differences in morphological appearance between juvenile and adult stages which is called heteroblasty (Goebel, 1898 in Allsopp, 1965). The morphological alteration in leaves is the most obvious characteristic of heteroblasty in vascular plants (Allsopp, 1965). Furthermore, Allsopp (1967) noted that leaf modifications in shape, size, anatomy and physiology often took place in heteroblastic development. Leaf shape is controlled by intrinsic factors for the basic pattern of ontogenetic change. This means that leaf shape is genetically determined, but environmental factors could reverse or slow down the transition direction.

Although heteroblasty has been observed since the 19th century, there is no fixed understanding of this term, except when adult leaves are present the formation of reproductive structures can take place (some heteroblastic species may not exhibit this phenomenon). Some people distinguish juvenile and adult leaves on the basis of subtle morphological differences, which is usually categorized as leaf plasticity (*e.g.* colour, size, thickness). Allsopp (1965) described heteroblasty in simple language, i.e. that the difference between juvenile and adult leaves is the occurence of gradual ontogenetic changes in certain type of leaves and a fairly sharp distinction in shape, especially when the adult leaves show strong modification *e.g.* into phyllodes, spines, or tendrils.

It has generally been accepted that the leaf is one of the aerial parts that receives direct environmental effects from its habitat. The environmental factors, *i.e.* photoperiod, light intensity, temperature, nutrition, availability of water, prevailing winds and gravity, will influence the ultimate size and/or the final shape achieved by adult leaves. However, it is still not certain whether the environmental factor directly affects the leaf primordia development themselves, or indirectly influence the physiology of entire plant development which then becomes evident in the ultimate dimensions of the adult leaves (Bostrack, 1993).

Allsopp (1954) and Crotty (1955) reported that there is correlation between the dimensions and/or organisation of the shoot apex with the characters of leaves developed from that apex. Several investigators provide evidence supporting this finding. Abbe *et al.* (1941), for example, found that increase in the dimensions of the

shoot apex in diploid corn was directly correlated with the increase in relative width of leaves 6 - 12. An inverse relationship was reported by Bruck and Kaplan (1980) in their study with *Muehlenbeckia* sp. In this genus, reduced early maturing scale leaves developed from larger shoot apices, while the later leaves which were larger, developed from smaller apical shoot meristems. Similar observation was carried out on *Fraxinus pennsylvanica, Tilia americana, Populus deltoides* and *Ulmus americana.* The results showed that there were no significant differences between the dimensions of apical meristems of sucker and crown branches of the first three species. Whereas in *Ulmus americana,* height and width of the apical meristems of crown branches were significantly smaller than those of sucker branches (Bostrack, 1993).

The role of environment on the morphological alteration of leaves has been widely observed. Several reports suggested that morphologically and anatomically, shade leaves exhibited larger surface areas, smaller transectional dimensions, thinner cuticles, fewer trichomes per unit area, proportionately more intercellular space within mesophyll tissue and fewer stomates per unit area of leaf surface than sun leaves (Wylie, 1951; Chaney and Kozlowski, 1969; Nobel *et al.*, 1975; Osborn and Taylor, 1990).

Moreover, Bostrack (1993) reported that leaves growing on the outermost periphery of the leaf crown usually have smaller surface areas than those growing in the inner parts of the leaf crown. This phenomenon is especially evidenced in branches that grow in the base of the trunk (Bostrack, 1993). However, there is also some contradictory evidence. Mature, shaded leaves of *Cornus florida rubra* and *Acer platanoides,* for example, display smaller surface areas than the exposed leaves (Isanoggle, 1944). This finding, however, may be explained by Blackman's (1956) investigation that at 50% daylight most leaves increase their surface areas, but, the expansion of shaded leaves falls if the light intensity is less than 50% of daylight (Bostrack, 1993).

According to some investigators, (Allsopp, 1954, 1965, 1967) nutrition seems to control heteroblasty. The level of available carbohydrates as well as varying environmental factors, *i.e.* photoperiod and temperature, could determine the leaf type being produced. Other findings suggested that certain chemicals are responsible for the formation of juvenile leaves (Frank and Renner, 1956).

Anatomical changes in heteroblasty were observed by Zalenski (1904, in Allsopp (1965)). It was reported that the subsequent leaves have xeromorphic characters

which are indicated by an increasing number of leaf veins per unit area, and gradual reduction in epidermal and mesophyll cell size. This finding was introduced as Zalenski's Law, and other investigators continued to support the validity of this law (Allsopp, 1965).

Research in heteroblasty has been conducted by many investigators and covers a wide range of problems and subjects. Most of them were carried out by qualitative morphological observations. Rumball (1963) conducted research on quantification of wood characters of *Podocarpus dacrydioides* and *Elaeocarpus hookerianus* in relation to heteroblastism. Although much work has been done to date, more studies in quantitative anatomy of heteroblastism needs to be carried out to ascertain the occurrence of structural alteration in heteroblasty development. The present research attempts to investigate the existence of the transition in stomatal protection mechanism between juvenile and adult leaves of *Banksia blechnifolia, B. marginata* and *Grevillea pyramidalis.* It is assumed that gross morphological differences between the two leaf types reflect the differences in the given mechanisms. In order to obtain representative data for this research, qualitative and quantitative observations were made.

3.2. Materials and methods

3.2.1. Sampling

Juvenile and adult leaves of two species of *Banksia* and *Grevillea pyramidalis* were sampled, in order to observe whether there were structural differences between them. Some juvenile leaves were harvested from glasshouse grown plants, the others were obtained from different localities. Because of time constraints, adult leaves were not harvested from glasshouse grown plants. They were sent from the National Botanical Gardens, Canberra, the Royal Botanical Gardens, Cranbourne and a *Grevillea* collector in Coraki, New South Wales.

Juvenile and adult leaves from two species of *Banksia* (*i.e. B. blechnifolia* and *B. marginata*) and *G. pyramidalis* were sampled from several places in Australia (Table 3.1, 3.2). Five leaves of both juveniles and adults were used as replicates. They were processed in the same way as outlined in Chapter 2 (see resin embedding, cuticle and ESEM preparation). Ten counts per leaf were applied in order to eliminate structural variety in the leaf samples.

Table 3.1. Sources of juvenile and adult leaves of *Banksia* and *Grevillea* observed.

Species	Source of leaves	
	Juvenile	Adult
B. blechnifolia	Plant Science glasshouse	Royal Botanical Gardens Cranbourne
B. marginata	Tasmania University campus	Tasmania University campus
G. pyramidalis	Plant Science glasshouse	Coraki, NSW

Table 3.2. List of *Banksia* species and *Grevillea pyramidalis* sampled with habitat and distribution in Australia (After George, 1994)

Species	Habitat	Distribution
B. blechnifolia	In white sand, in heath or mallee heath. Annual rainfall 400 mm.	Western Australia; between Jerramungup and Gibson.
B. marginata	In sandy loam, clay loam, shale, peaty loam and rocky soil (quartzite, sandstone, limestone and granite); occurs in shrubland, woodland and forest, sometimes in swamps and on coastal dunes. Annual rainfall 400-1000 mm.	South-eastern Australia from Eyre Peninsula, South Australia, through Victoria and eastern New South Wales north to Baradine and Guyra; throughout Tasmania; also on Kangaroo Island and the islands of Bass Strait. Often locally common.
G. pyramidalis	In open eucalypt woodland or savannah, and *Triodia* communities; sandy soil on sandstone, limestone, slate, bauxite.	North-western Australia. In Western Australia, from about the Hammersley Ranges to the Eastern Kimberleys (with one station only in the Great Sandy Desert), Northern Territory, North of c. 14°S

3.2.2. Resin embedding section

Both juvenile and adult leaves were sectioned. Details on resin embedding section protocol are outlined in Chapter 2.

3.2.3. Cuticle preparation for transmitted light microscopy

Some *Grevillea* leaves had thinner cuticle than *Banksia* species. Even though a procedure similar to that applied to *Banksia* (see Chapter 2) was applied, careful handling was necessary.

3.2.4. SEM preparation for morphological observation

Some *Grevillea* species have needle-like leaves. In this case there is a real probem positioning the leaves, because the stomates are covered by the recurved margins ESEM preparation details were outlined in Chapter 2.

3.2.5. Data collection

Data were collected for the selected characters, which were obtained from the two leaf forms, counted and measured. Special characters that were not quantified were considered important as supporting data.

3.2.5.1. Quantitative data

The methods of counting and measuring characters were similar to those in Chapter 2. However, every species had two leaf forms that were considered as separate treatments. Two data sets for every species were analyzed and tested, in order to determine the differences between the two leaf forms.

3.2.5.2. Qualitative data

Qualitative data were very important in supporting the phenomena of structural stomatal protection mechanisms in the transition from juvenile to adult leaves, especially for the following qualitative characters:

- leaf shape and margins;
- the presence of hair clumps; and
- the presence of cuticle projections.

3.2.6. Data analysis

For the two *Banksia* species, data obtained were analysed by Student *t*-test to determined significant differences between juvenile and adult leaf characters. Meanwhile, for *G. pyramidalis*, the data were analysed by a one-way analysis of variance. Subsequently, Tukey's procedures with $p = 0.05$ were used to separate means of every given character of seedling, juvenile and adult leaves.

3.3. Results

3.3.1. *Banksia blechnifolia*

The results of observations of leaf cross sections and ESEM images of the upper epidermis are presented in Figure 3.1A-D. Hairs and stomatal depressions are the most prominent characters found in both juvenile and adult leaves. In juvenile leaves, wavy and thick hairs were distributed densely on the epidermis, while slender and spiral hairs appeared to occlude the surface of the depression. The presence of hairs seemed to change as the plant progresses toward maturity. Adult leaves (Figure 3.1C,D) were not covered by dense epidermal hairs but the presence of hair bases indicates the existence of hairs at some stage of adult leaf development. The hair structure around depression surface areas in adult leaves was very much similar to that on juvenile leaves, but they appeared to be denser. Unfortunately, due to technical difficulties, no quantitative measurements were made to support this suggestion.

Differences in depression characteristics between juvenile and adult leaves are clearly shown in Figure 3.1A,C. The depressions of adult leaves are deeper and narrower than those of juvenile leaves. The quantitative observation emphasized these facts in which depression depth (dd) and the dimension of surface area (ws and ls) and median area (wm and lm) showed highly significant differences between leaves at different stages of development. These differences may be well summarized by significantly different values of index deppression (Id). However, it is interesting to note that the shape of the surface area of a depression, as indicated by the ratio of ws and ls, did not change significantly with growth phase alteration. In both leaves, the depression surface area tends to be elliptical in shape.

Another prominent character that differentiates juvenile and adult leaves is the upper epidermis cuticle (Uec). It seems that this character plays a significant role in successful plant development, so that mature leaves tend to develop much thicker cuticle than juvenile leaves (Table 3.3).

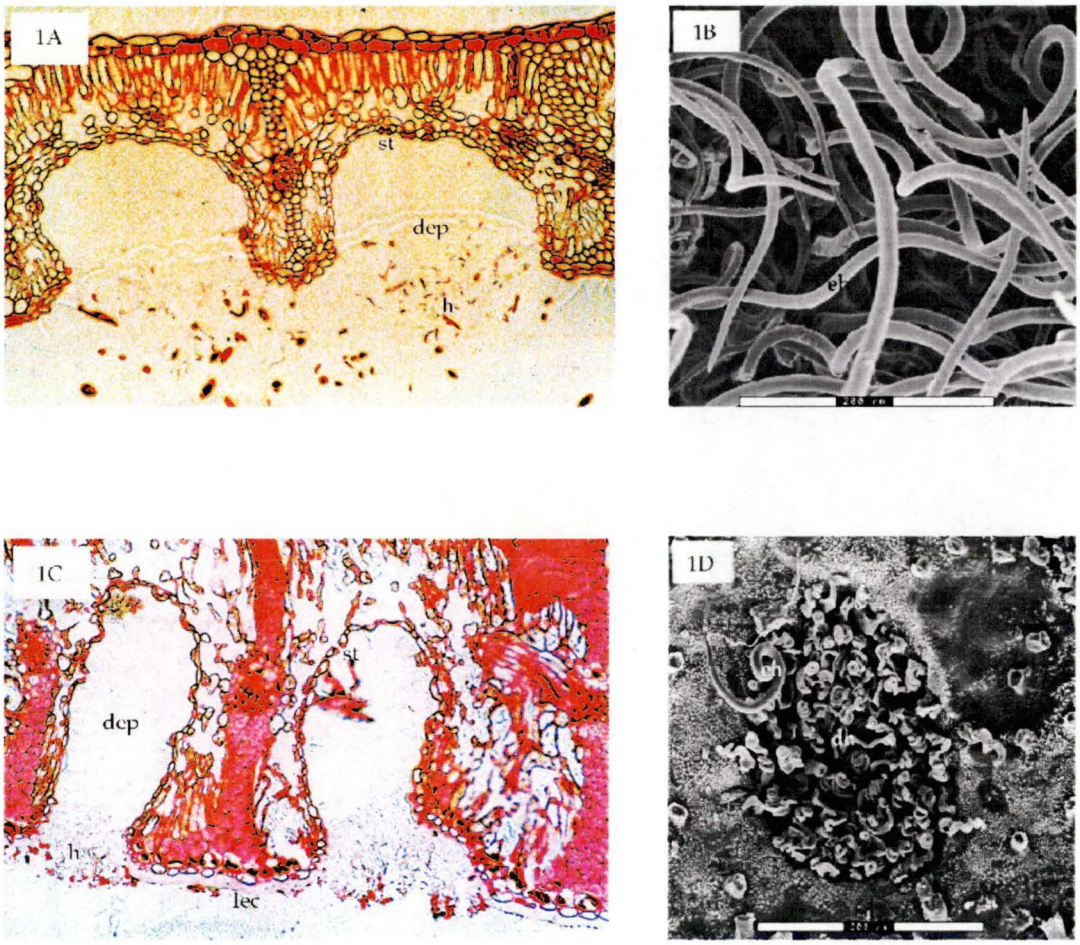


Figure 3.1A-D. Cross sections of seedling (1A, 160x) and adult (1C, 160x) leaves of *B. blechnifolia*. Note that the stomatal depressions develop into more intensive shapes, deeper and narrower (1A, 1C). In seedling leaves (1B), epidermis hairs (thicker) are prominent, they thus predominate over the depression hairs. In the adult leaves (1D), depression hairs are conspicuous and dense and they occlude the surface area of stomatal depressions. Epidermis hairs are less dense than in the seedling leaves.
dep = depression; dh = depression hairs; eh = epidermis hairs; h = hairs; st = stomate.

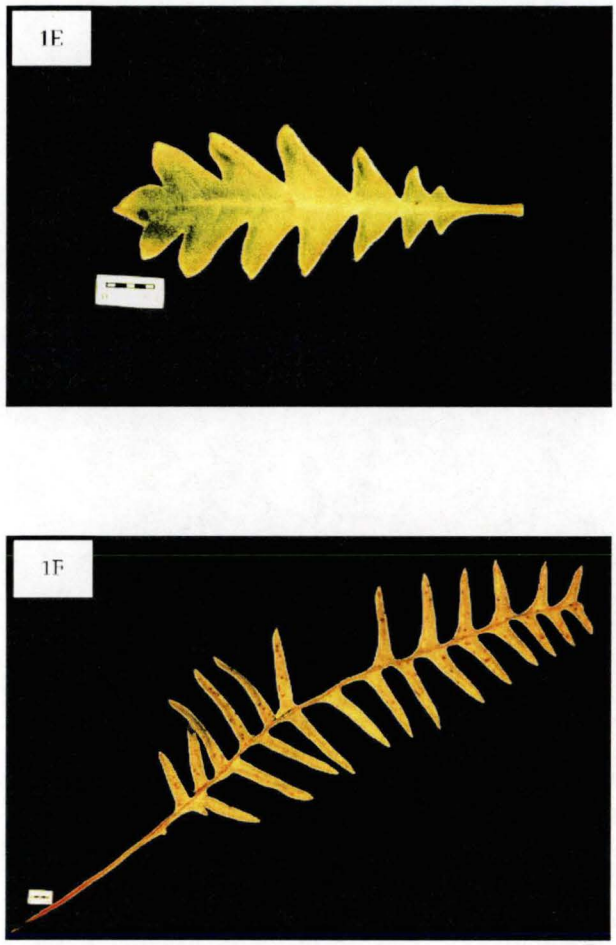


Figure 3.1E and F. Showing seedling and adult leaves of *B. blechnifolia*. The seedling leaf is deeply lobed, with narrowly triangular and entire margined lobes. The adult leaf presents similar features, however it has linear lobes. Scale bar = 1 cm.

Table 3.3. Character values and t-test results for juvenile and adult leaves of *B. blechnifolia.*

Characters	Juvenile leaf	Adult leaf	*t*-test	Pobability
Depression				
dd	200.73	392.93	21.658	< 0.0001
ws	140.03	122.00	3.171	0.0132
ls	244.73	198.45	4.842	0.0013
ws/ls	0.59	0.64	1.815	0.1071
wm	163.83	240.64	15.350	< 0.0001
lm	268.65	320.45	5.975	0.0003
wm/lm	0.62	0.77	6.520	0.0002
Id	2.61	10.49	19.442	< 0.0001
Cuticle				
Uec	2.58	15.76	22.893	< 0.0001

3.3.2. *Banksia marginata*

Figure 3.2A-D depict leaf cross sections and ESEM images of the upper epidermis of juvenile and adult leaves of *B. marginata.* In general, there is no appreciable difference in the characteristics of juvenile and adult leaves. Depression and hair structures in both leaves developed similarly, but slightly denser hairs were observed in juvenile leaves. Meanwhile Figure 3.2E indicates that there is a different pattern of leaf development between the juvenile and adult phase. The juvenile leaf is oblong with serrate margin, whereas the adult is oblong with an entire margin.

Quantitative observation provides more detailed results. It was found that the depression index of adult leaves was significantly higher ($p<0.02$) than the juvenile one. However, this difference was mainly caused by the depression surface area dimensions (ws and ls) that tends to be proportionally smaller in adult leaves.

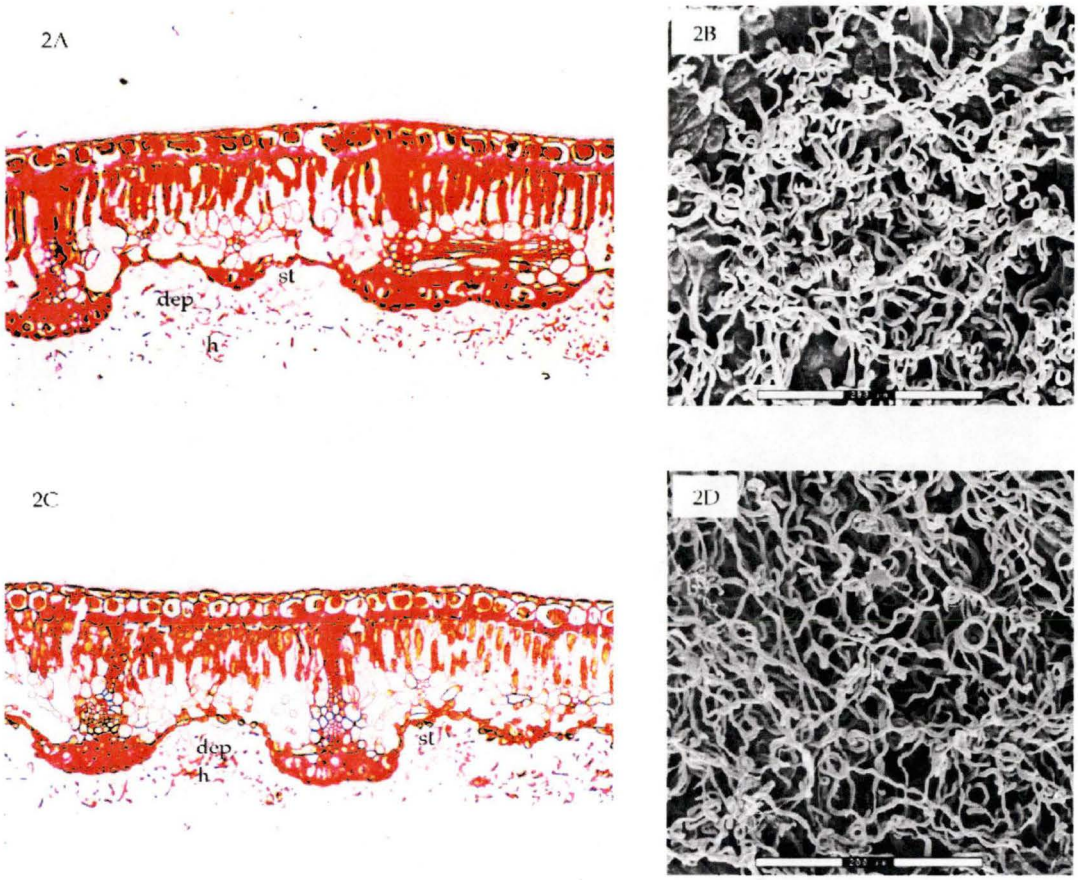
Although no significant difference was found in the hair dimensions (ldh and ddh), the value of hair index (Ih) of the adult leaf was considerably higher ($p< 0.05$) than that of the juvenile leaf. This was mainly because of the higher hair density (dh) observed in the adult leaves. The two types of leaves have similarities in the

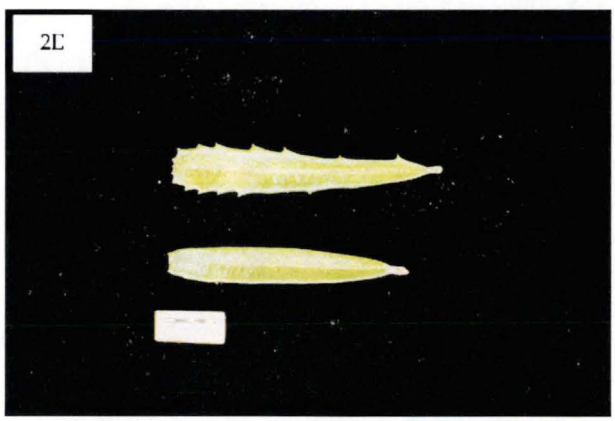
epidermis index (Ie), as the result of similar stomate density (ds) and in the thickness of the stomatal and upper epidermis cuticle (Sc and Uec). Yet, they differ marginally in the thickness of the lower epidermis cuticle.

In summary, therefore, both qualitative and quantitative observations suggested that in spite of the apparent differences in the pattern of the gross leaf morphology , there is little evidence of a structural transition occuring in this species.

Table 3.4. Character values and t-test results for juvenile and adult leaves of *B. marginata*

Characters	Juvenile leaf	Adult leaf	*t*-test	Pobability
Depression				
dd	92.91	89.31	0.454	0.6619
ws	175.49	144.96	3.820	0.0051
ls	336.56	296.77	2.240	0.0554
ws/ls	0.53	0.51	0.664	0.5254
wm	115.27	103.25	1.776	0.1137
lm	276.46	252.78	1.297	0.2308
wm/lm	0.43	0.43	0.105	0.9191
Id	0.70	0.80	2.916	0.0194
Hair				
ldh	1046.94	968.21	1.620	0.1440
ddh	15.51	14.48	1.304	0.2286
Ih	18272.80	14864.05	2.852	0.0214
Epidermis				
ds	31.58	29.88	1.339	0.2172
dh	40.56	38.28	2.393	0.0437
Ie	1.29	1.31	0.470	0.6507
Cuticle				
Sc	0.75	0.75	-	-
Lec	2.73	2.46	2.427	0.0414
Uec	4.61	4.24	1.703	0.1270





Figures 3.2A-D. Cross sections of juvenile (2A, 160x) and adult (2C, 160x) leaves of *B.marginata*. It seems that there is no difference between depressions in juvenile and adult leaves of *B. marginata*, both of which have a similar level of depressions. However, in the adult leaves (2D), hairs grow more abundantly than in the juvenile (2B) leaves.
dep = depression; h = hairs; st = stomate.

Figure 3.2E. Showing juvenile (above) and adult (below) leaves of *B. marginata*. The juvenile leaf is oblong with a serrate margin, whereas the adult is oblong with an entire margin. Scale bar = 1 cm.

3.3.3. *Grevillea pyramidalis.*

It was possible to observe three different stages of development in this species, namely seedling, juvenile and adult phases. Figure 3.4A-F and 3.4G-I illustrate the differences in both anatomical and morphological structures between them. Seedling leaves are single bifacial and have conspicuous midribs. Juvenile leaves are pinnatisect with bifacial and unifacial parts, whereas adult leaves are pinnatisect with unifacial surface. The leaf cross sections showed that stomatal positions develop from exposed in seedling to gradually hidden in the next phase of development. Similarly, the hair structures become more numerous with the phase transition. Seedling leaves are characteristized by a small number of hairs, while the adult leaves are covered with a highly dense hair structure.

Table 3.5. Character values and t-test results for juvenile and adult leaves of *G. pyramidalis*

Characters	Seedling leaf	Juvenile leaf	Adult leaf	*F*-test	Probability
Depression					
dd	-	55.89	118.64	16.515	< 0.0001
dw	-	67.07	41.23	5.894	0.0004
Id	-	0.90	3.56	5.454	0.0006
Hair					
lhp	879.98 *a**	855.46 *a*	916.16 *a*	1.948	0.1851
dhp	22.36 *a*	22.16 *a*	15.15 *b*	7.069	0.0094
lvp	24.70 *a*	29.75 *a*	29.51 *a*	0.950	0.4138
dvp	35.28 *a*	43.39 *a*	28.43 *b*	9.417	0.0035
Ih	47747.52 *a*	268152.01 *b*	339283.32 *b*	36.250	< 0.0001
Epidermis					
ds	19.48 *a*	34.98 *b*	31.75 *b*	37.837	< 0.0001
dh	3.12 *a*	13.78 *b*	23.00 *c*	121.652	< 0.0001
Ie	0.18 *a*	0.42 *b*	0.73 *c*	38.066	< 0.0001

* Mean separation using Tukey's procedures (p = 0.05)

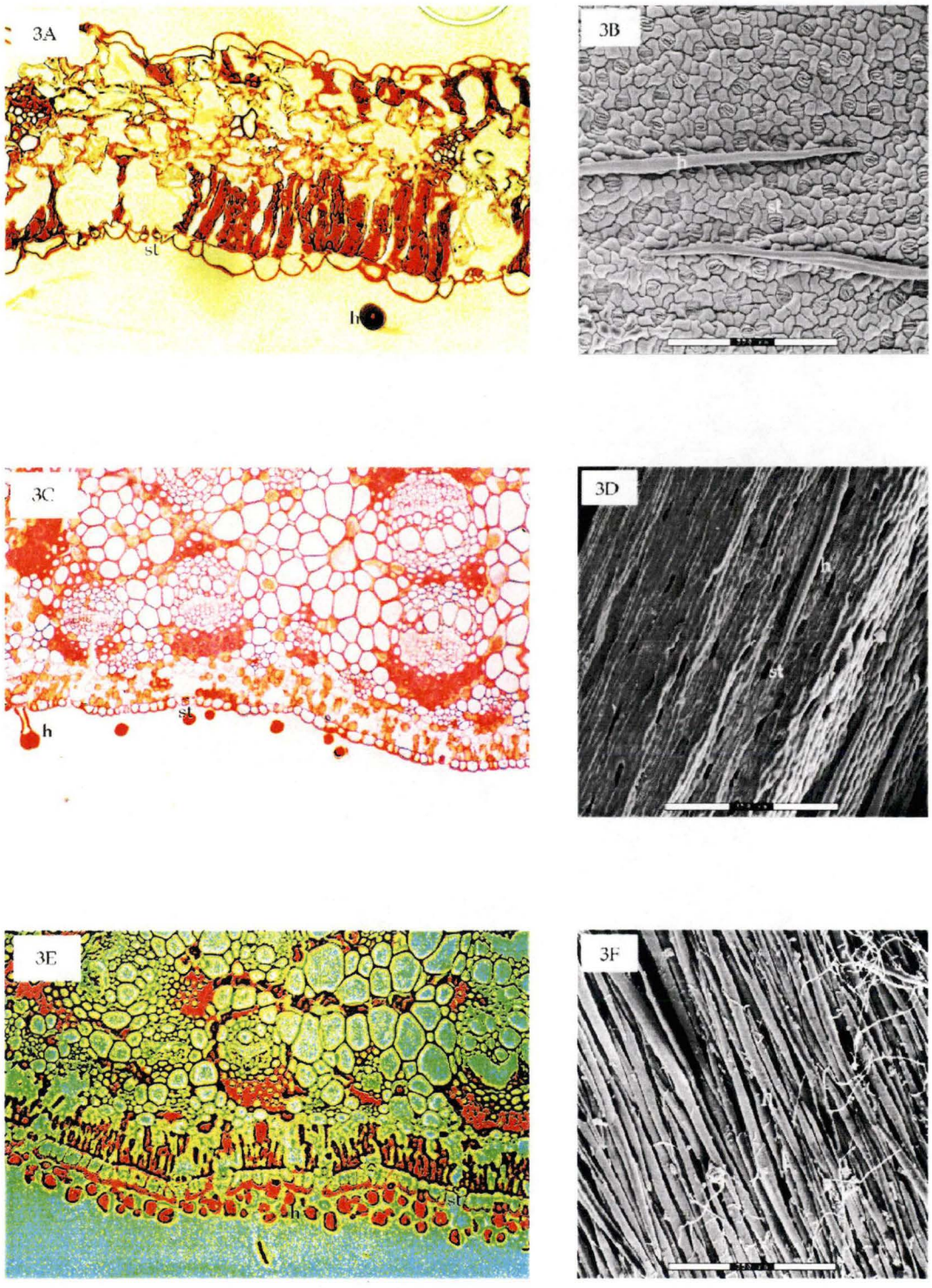


Figure3.3A-F. Cross sections of seedling (Fig. 3A, 320x), juvenile (3C, 160x) and adult (3D, 160x) leaves of *Grevillea pyramidalis*, respectively. Stomatal positions subsequently develop from exposed to hidden positions (Figures 3 B, D, F). The arrangement of hairs also continuously develop from rare to very dense.
ct = cuticles; h = hairs; st = stomates.

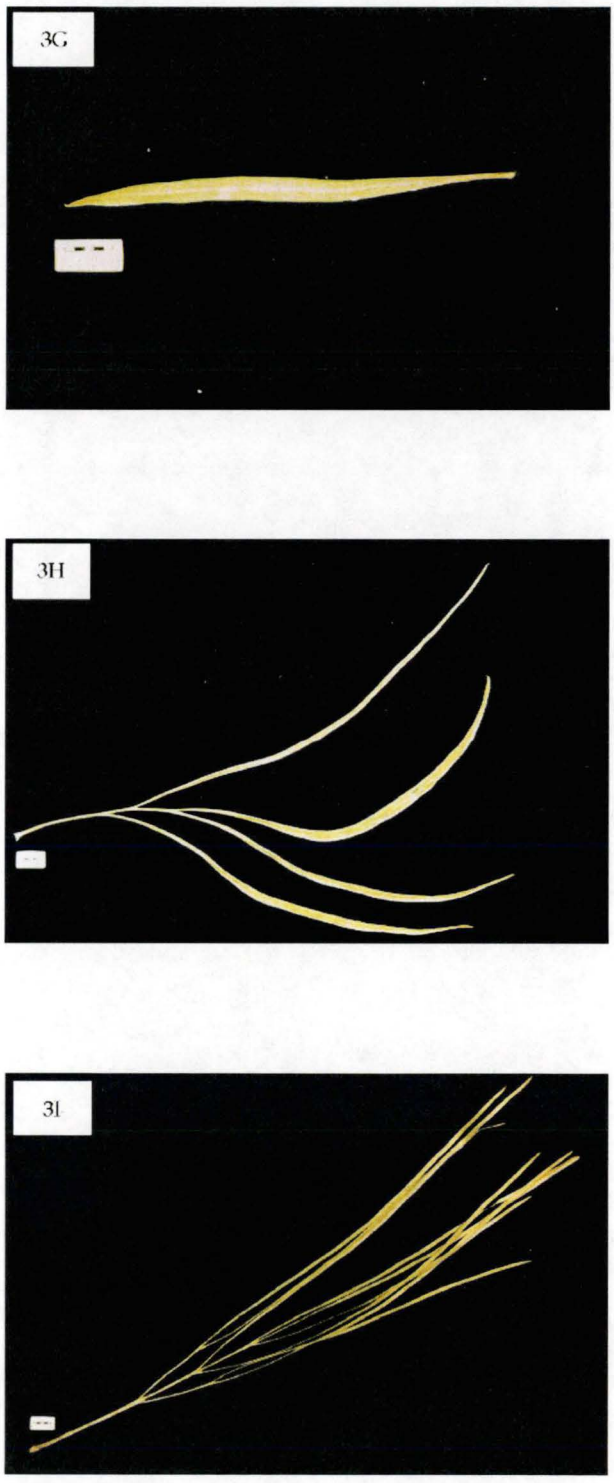


Figure 3.3G-I. Leaves of *G. pyramidalis* develop from seedling to adult leaves. Seedling leaves (G) are single bifacial and have conspicuous midribs. Juvenile (transition) (H) leaves are pinnatisect with bifacial and unifacial parts. Adult leaves (I) are pinnatisect and unifacial. Scale bar = 1 cm.

The quantitative observations confirmed these suggestions. It was found that both the depth and diameter of the depression increased significantly ($p<0.001$) as development phase progressed toward maturity. This means that the transition produces more protected stomates.

Increasing stomatal protection in more advanced leaves is also gained by increasing hair density, but not by hair dimensions which is found to be thinner ($p<0.01$) for both vertical and horizontal parts. However, the hair length seemed to be similar in all phases of development. The importance of the role of hairs in stomatal protection is reflected by a significant increase in the values of hair index in more advanced leaves.

It is interesting to note that the increase of the component of stomatal protection is accompanied by the increase in stomatal density. This may link to an increase in physiological activity in the adult plants.

3.4. Discussion

Plant adaptation to different environments could be reflected by the formation of two different developmental stages, namely juvenile and adult phases. Naturally, the juvenile phase is more adaptable to abundant water conditions while the adult phase tends to adapt to conditions with less water (Allsopp, 1967). This suggestion is clearly exemplified by general plant growth, that seedlings usually grow under the protection of the adults from excessive radiation, wind run and temperature, therefore ensuring moisture avaibility. However, as they reach maturity, they tend to be exposed to open and drier environments.

This is supported by the results of this research, which shows that there is a general trend of increasing stomatal protection among species observed as the plants move into more advanced stages of development. Such stomatal protection increases could be needed to cope with harsher conditions. Characters which are mostly observed to alter during phase transition are depressions and hairs.

Depressions

Three different types of stomatal depression were found. In *Banksia blechnifolia,* the juvenile leaf has relatively shallow and wide depressions, and the adult leaf develops depressions that are deeper and slightly narrower in diameter, forming a slender "balloon-like pit" (Hill, 1994). In such a depression, stomates are very hidden, and thus very protected from excessive loss of moisture (Chapter 2). The second pattern is shown by leaves of *Grevillea pyramidalis.* Three stages of depression development occur in this species. This supports the assumption of Hill (1994) that there is a range of leaf depressions as the climate becomes drier. However, different features were found in *Banksia marginata.* There is no alteration of the size and shape of the depression in this species. Presumably, *B. marginata* has other characters that can take over the role of stomatal protection. It is possible that many different characters make small contributions to stomatal protection.

Hairs

Hairs seem to be important in *Banksia* and *Grevillea,* because they are always present in large numbers. However, statistical analysis does not provide a convincing association between hairs and environmental factors.

In *B. blechnifolia,* there are two types of hairs, namely epidermis and depression hairs. At the juvenile stage, epidermis hairs predominate over depression hairs in protecting the stomates. As the leaves make the transition to adult, leaf stomatal protection is taken over by depression hairs that develop into a denser arrangement. Moreover, the spiral structure of depression hairs increase their impenetrability. In this case, due to the very dense hairs that occlude the mouth of depressions, the stomates inside are securely protected from the harsh environment. In *B. marginata,* adult leaves have denser hairs than the juvenile. This character seems to increase the stomatal protection (and boundary layer) as the depression does not show any alteration. Hairs in *G. pyramidalis* show a gradual alteration in density from seedling through juvenile, and to adult leaves. Although adult leaves have more slender hairs than seedling leaves, the density is much higher, giving a better coverage of the leaf surface.

Leaf shape

Gross morphology of leaves is also an important indicator of plant adaptation. *Banksia blechnifolia* has similar leaf shapes both in juvenile and adult stages, *i.e.* a deeply lobed shape. An insignificant difference is in the shape of lobes, in juvenile leaves they are narrowly triangular, but in adults they are linear lobed (Figure 3.1F).

3.5. Conclusion

The results of this study support the suggestion that phase transition during plant development is a part of the plant strategy to adapt to increasingly harsh environments as the plant grows to maturity. This involves alteration of the complexity of the leaf structures. Seedlings of the three species observed tend to have simpler stomatal protection mechanisms than do the adult leaves, since naturally they grow under the protection of the adult plants. As they approach maturity, they may be exposed to harsher conditions and, therefore, a more complex stomatal protection mechanism might help the adult plant to cope with the situation.

The leaf structural changes from the juvenile to adult phase varies among the species observed. In *Banksia blechnifolia* and *B. marginata,* stomatal depressions are found in both seedling and adult leaves. However, while the depression of *B. blechnifolia* develop into a more protective shape in the adult stage, no significant alteration is found in the depression of *B. marginata.* Unlike the two *Banksia* species, the leaves of the seedlings of *G. pyramidalis* do not exhibit stomatal depressions, but this character is eventually present in the more advanced stage of leaf development.

Hair characteristics are found to change with phase transition but the trend of this alteration is also species dependent. In *B. blechnifolia,* hairs are found abundantly covering the epidermis of the seedling leaves, but most of these hairs detach from the adult leaves leaving the hair bases on the epidermis. Instead of retaining epidermal hairs, the adult leaves tend to develop denser hairs that occlude the surface of the stomatal depression. The protective impacts of such a structure could be very effective in reducing the loss of moisture from the leaves. In *B. marginata* there is no significant alteration in hair structure from juvenile to adult phases. Meanwhile, in *G. pyramidalis* the alteration of hair structure is considerable. The density of hairs in this species significantly increases with advancing plant development, but the dimensions of hairs, especially their diameters, decrease.

There is no clear pattern to the alteration of stomate density and the thickness of the cuticle. In *G. pyramidalis* the density of stomates is higher in the more advanced leaves, while in *B. marginata* a relatively similar stomate density was observed throughout. Significant stomatal thickening during leaf phase change seems to be specific to *B. blechnifolia.*

Chapter 4

General conclusion

One important characteristic of living organisms is their ability to adapt to habitat conditions. They can, therefore, stay alive, reproduce successfully, and if there is a possibility to dominate the habitat they will do so. Some organisms are very plastic in responding to habitat conditions, however, others are less plastic or even very sensitive. Plants respond similarly to other organisms, but, because they are fixed in space, they are more structurally responsive. Among plant organs, the leaf is usually the most sensitive, since it covers the widest area of the plant, so that it is more exposed to the environment, and it is also the major site of photosynthesis, which is very temperature sensitive.

The Proteaceae are a family dominating the Australian vegetation. The members of this family spread all over the Australian continent and Tasmania (Wrigley and Fagg, 1991). They have adapted to the Australian climate, which is generally hot and dry, and the soil often has a low phosphorus content. The morphological appearance of Proteaceae species, thus, shows adaptive forms to these conditions. Until recently, the morphological characteristics of plants responding either to dry or low phosphorus soil contents has not been obviously separated (Hill and Brodribb 2001).

This present research has been carried out to investigate xeromorphic characters in *Banksia, Grevillea* and *Orites*. It was expected that the findings would assist in solving the problems of the xero-scleromorphy dichotomy. In order to provide a clear understanding of the whole research, the main aims will be briefly elaborated.

I. Morphological Range and Transition of Stomatal Protection Mechanisms in Some selected Proteaceae

The problems faced by plants growing in dry areas are coping with excessive transpiration, both through the stomates and cuticles. The genera *Banksia, Grevillea* and *Orites* are not CAM (Crassulasean Acid Metabolism) plants. Therefore, they can not avoid the opening their stomates for gas exchange during the day unless it is too dry for this to happen. Some of the structures present are very strongly developed, and thus are suspected to reduce or protect against excessive transpiration. Hill (1998) assumed that abundant hairs, stomatal depressions, and revolute leaf margins may increase the boundary layer in some Proteaceae, whilst a thick cuticle may reduce water loss (Cowan, 1977).

Among the three genera observed there was no general structure assumed to increase the boundary layers. In *Banksia* stomatal depressions and hairs are prominent, whilst in *Grevillea,* recurved leaf margins and hairs are obvious. *Orites* is an exception, since it does not show any structure assumed to increase the boundary layer.

The present results, however, demonstrate that the typical characters assumed to increase the boundary layer appear in every genus, but did not always show a significant correlation with the habitat. Their persistence, however, might have some role in coping with dryness since they have adapted for millions of years in such conditions and they may not currently be easy to correlate with thei habitat because species distributions may not have reached equilibrium since the last glaciation ended.

Stomatal depressions, which are mostly found in *Banksia,* are very likely to play a role in protecting the stomates from the physical factors of the environment, especially from temperature and radiation effects. During the life history of some species, as observed in *B. blechnifolia,* stomatal depression may play an important role in protecting the stomates. The intensive growth of this structure during maturity implies that, in the adult phase plants are more exposed to dry conditions, as it was assumed that during the juvenile state they are more protected or grow in less severe environments.

The persistence of hairs and leaf recurvation, especially in *Banksia* and *Grevillea,* is correlated with habitat climatic factors. Even though hairs are not a typical structure in *Orites,* species which possess this structure showed a strong correlation between hair dimension and densities to habitat climatic factors. Hairs were also found to grow intensively, particularly in *Grevillea pyramidalis,* into the adult stage. It is logical that growing into the adult phase the plant will experience harsher conditions, since during juvenile and seedling states they grow under the canopy of the adult plants or else grow during a wet phase. In *Grevillea,* the hairs grow denser and cover the stomates, hence the stomatal boundary layer increases. However, in *B. blechnifolia* the hairs grow denser and occlude the stomatal depression. In this case the stomates are seemingly protected by both stomatal depression and hairs.

The presence of thick cuticles, especially in *Grevillea,* may significantly reduce water loss. The habitat climatic factors displayed greater links to the thickness of stomatal and lower epidermis cuticles than to the upper epidermis cuticle. In this genus, also, hairs behave contradictory to that already hypothesised, the higher the minimum of the range of the annual rainfall, the greater the hair size.

In summary, although the plants observed possessed structures that are assumed to increase the boundary layer, statistically some of them do not show any link with the habitat climatic factors determined. There is a possibility that the living plants are

not in equilibrium with the prevailing climate. The most likely characters that could protect the stomates are stomatal depressions and hairs. This idea is in accordance with the life history traits of *B. blechnifolia* and *G. pyramidalis*, showing that these structures grow intensively in adult leaves.

II. Possible Evolution of Stomatal Protection Mechanisms in *Banksia*

The present Australian climate is hot and dry, especially in the inland areas. Some coastline areas, however, have enough rain for complex forest to grow. However, the Australian climate has not always been like this. The rapid drifting of the Australian continent to lower latitudes impacted on Cainozoic climates. During the Palaeogene (~65 – 25 myr ago) high humidity and temperature persisted. At the end of the Eocene (about 35 myr ago) when the Australian continent was still in higher latitudes, it experienced warm and wet climates. Frakes (1999) assumed that the global drying started about 25 – 35 myr ago. However, until about 11 myr ago relatively high rainfalls occurred, followed by a widespread drying (Hill and Brodribb, 2001).

The present Australian climate has recent origins. The vegetation of the Australian continent, thus, has responded to climate change by altering its structure. The plants responses can be investigated by observing the plant macrofossil records and living plants.

The phenomenon of scleromorphism (thick leaves with dense venations) was found in the oldest Banksieae macrofossils, *Banksieaephyllum taylorii*, from late Paleocene sediments in south-eastern New South Wales (Carpenter, *et al.* 1994). Other morphological features showed that the non stomatal surface was striated, possibly functioning to channel water off the leaf surface. Moreover, on the stomatal surface hairs persisted, which also possibly function to trap water and stop it reaching the stomatal pores. This is in accordance with the wet prevailing climate reported by Taylor, *et al.* (1990).

Similar cuticular striations were also investigated in *Banksieaephyllum* species that appeared in several mid Eocene macrofloras in south-eastern Australia (Hill and Christophel, 1988). Hill (1998) described typical striations found on one or both surfaces as often radiating away the stomates. Moreover, the cuticular ledges protecting the pores from flooding grow intensively (Hill, 1998). This evidence indicates that the habitat conditions were quite wet, so that plants need to protect the stomates securely (Hill and Brodribb, 2001).

From the late Eocene Kojonup flora from Western Australia, McLoughlin and Hill (1996) reported that many Banksieae taxa have deep stomatal depressions and revolute leaf margins. The presence of stomatal pits and hairs are also displayed by the macrofossils found in the Latrobe Valley coal. The presence of hairs in the eight species of *Banksieaephyllum* macrofossils found in this area are assumed to have double functions, as water trappers and water protectors (Cookson and Duigan, 1950; Hill, 1990). Two species of *Banksieaephyllum* namely, *B. angustum* and *B. acuminatum* displayed xeromorphic structures with well-developed leaf margin recurvations, stomatal depressions and dense trichomes (Blackburn, 1985). Furthermore, Hill (1998) proposed that the presence of structures increasing the boundary layers is strong evidence supporting the phenomenon of low water availability during the time of their appearance.

Jordan and Hill (1991) also reported that some more recent macrofossil records showed well-developed functional boundary layers, *e.g.* the presence of deep stomatal depressions in the Pleistocene *B. kingii*; and very narrow revolute leaf margins in the Early Pleistocene *Banksia strahanensis* from the west coast of Tasmania.

In summary, developing stomatal protection mechanisms investigated in the macrofossils records, provides supporting evidence of increasing aridity in the Australian continent starting between 24 and 35 myr ago. Similar responses are displayed by the living banksias. *Banksia* species inhabiting drier areas show more well-developed structures increasing the boundary layers than those living in less dry areas. Statistically, it has been shown that there is a significant link between increasing the boundary layer and aridity. Based on this finding a reconstruction of the stomatal protection mechanisms in *Banksia* is described as follows (Figure 4.1), represented by anatomical details of living plant leaves.

Reconstruction of the Stomatal Protection Mechanisms in *Banksia*

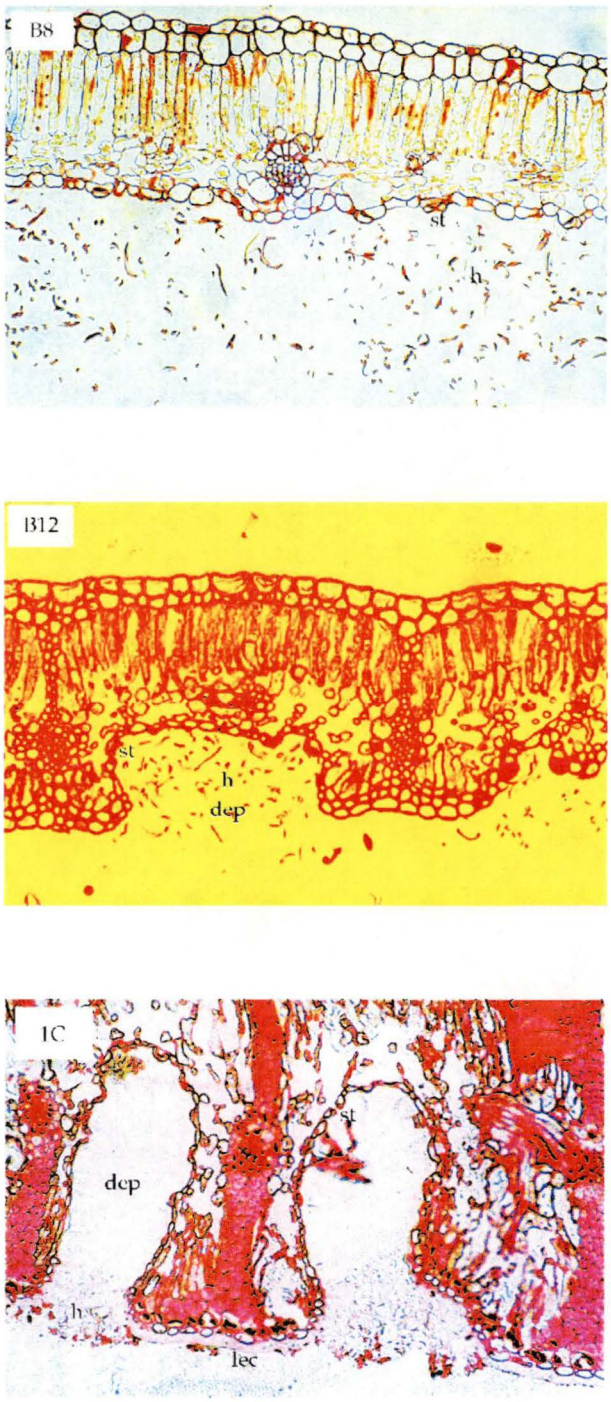


Figure 4.1. Showing the development of stomatal depressions into the more intensive stomatal protection strategy, represented by *B. occidentalis* (160x-upper), *B. serrata* (160x-middle) and *B. blechnifolia* (160x-lower). Stomatal depressions in *B. blechnifolia* show an extreme protection with very dense hairs occlude the stomatal depression surfaces. dep = stomatal depression; h= hairs; lec = lower epidermis cuticles; st = stomate.

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